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(54) Title: SELF-ASSEMBLING GENES, VECTORS AND USES THEREOF		
(57) Abstract		
<p>The invention relates to a method for directing the self-assembly of a gene or gene assembly having three and preferably six or more fragments in a directionally and spatially ordered fashion to produce a gene, gene vector or large nucleic acid molecule. The method can be used to create libraries, such as combinatorial libraries. In another embodiment of the invention a vector is described for the incorporation and screening of endogenous mouse promoter elements for the identification of cell-specific promoters.</p>		

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**SELF-ASSEMBLING GENES, VECTORS AND USES THEREOF****Field of the Invention**

5 This invention relates to the construction and usage of synthetic genes for genetic engineering and gene therapy.

**Background of the invention**

This application claims the benefit of a provisional application U.S. Serial No. 10 60/070,910, filed on February 28, 1997, entitled "Self-Assembling Genes."

Recombination at the genetic level is important for generating diversity and adaptive change within genomes of virtually all organisms. Recombinant DNA technology is based upon simple 'cut-and-paste' methods for manipulating nucleic acid molecules *in vitro*. The pieces of genetic material, or DNA are first digested with a restriction endonuclease

15 enzyme which recognizes specific sequences within the DNA. After preparation of two or more pieces of DNA, the ends of the DNA are further manipulated, if necessary, to make them compatible for ligation or joining together. DNA ligase, together with adenosine triphosphate (ATP) is added to the genes, ligating them back together. The genetic assembly containing an origin of DNA replication and a selectable gene is then inserted into a living 20 cell, is grown up, and is positively selected to yield a pure culture capable of providing high yields of individual recombinant DNA molecules, or their products such as RNA or protein.

Significant improvements have been made to this technology over the last two and a half decades. Numerous enzymes, end-linkers and adapter molecules have been made commercially available, which facilitate in the construction of recombinant DNA molecules.

25 By using two restriction enzymes with different single-stranded termini or blunt ends, it is possible to directionally assemble genes (forced cloning). This reduces the amount of screening required to determine orientation. Procedures have been automated for synthesis of single-stranded gene fragments up to 200 or more nucleotides in length by means of phosphoramidite chemistry, and the instrumentation is readily available through Applied 30 Biosystems, Inc., Foster City, CA. Such single-stranded fragments can be joined by annealing overlapping complimentary phosphorylated strands, and by enzymatically filling in the ends with DNA polymerase and DNA precursors. In this way, multiple, overlapping, single-stranded fragments can be assembled into a larger, double-stranded superstructure.

Whole genes have been synthesized by similar methods. However, it becomes increasingly difficult to use synthetic DNA strands when making genes larger than approximately one kilobase. Using gene amplification methods (e.g. polymerase chain reaction (PCR), Mullis *et al.*, U.S. Patent 4,683,195), together with synthetic oligonucleotides, it is possible to make 5 biologically active, synthetic retro-vectors that are capable of RNA transcription, reverse-transcription, viral packaging, and integration into genomic DNA (see for example, Hodgson, WO94/20608). Hodgson, *supra*, also disclosed methods for cloning of transcriptional promoters into such a vector using traditional recombinant DNA technology.

Modified restriction enzyme sites, linkers, and adapters can change the 10 primary or secondary structure of complex nucleic acid sequences thereby altering or obliterating a desired biological activity. For example, small mutations can drastically modify transcriptional promoters or change the reading frame of coding DNA. A logical goal of vectorology is to make exact constructs, without need of fortuitous restriction sites, adapters, or linkers.

15        Restriction endonucleases can be grouped based on similar characteristics. In general there are three major types or classes: I, II (including IIS) and III. Class I enzymes cuts at a somewhat random site from the enzyme recognition sites (see Old and Primrose, 1994. *Principles of Gene Manipulation*. Blackwell Sciences, Inc., Cambridge, MA, p.24). Most enzymes used in molecular biology are type II enzymes. These enzymes recognize a 20 particular target sequence (i.e., restriction endonuclease recognition site) and break the polynucleotide chains within or near to the recognition site. The type II recognition sequences are continuous or interrupted. Class IIS enzymes (i.e., type IIS enzymes) have asymmetric recognition sequences. Cleavage occurs at a distance from the recognition site.

These enzymes have been reviewed by Szybalski *et al.* *Gene* 100:13-26, 1991. Class 25 III restriction enzymes are rare and are not commonly used in molecular biology.

U.S. Patent No. 4,293,652 employed a linker with a class IIS enzyme 25 recognition sequence to permit synthesized DNA to be inserted into a vector without disturbing a recognition sequence. Brousseau *et al.* (*Gene* 17:279-289, 1982) and Urdea *et al.* (*Proc. Natl. Acad. Sci. USA* 80:7461-7465, 1983) disclose the use of class IIS enzymes for 30 the production of vectors to produce recombinant insulin and epidermal growth factor respectively. Mandecki *et al.* described a method for making synthetic genes by cloning small oligonucleotides using a vector (*Gene* 68:101-107, 1988). Expansion of a population of

oligonucleotides required synthesis, cloning excision and fragment purification. The oligonucleotides were used to create a complete plasmid.

Lebedenko et al. (*Nucl. Acids Res.* 19(24):6757-6771) illustrated the class IIS enzymes and PCR for precisely joining 3 nucleic acid molecules for convention sub-cloning  
5 using BamHI. Tomic et al. (*Nucleic Acids Res.*, 18:1656, 1990), reported a method for site-directed mutagenesis using the polymerase chain reaction and class IIS enzymes to join two nucleic acid molecules. Two overlapping PCR primers were used where the primers included class IIS recognition sites. The primers included a region of complementarity to the template DNA and include one to a few site-directed mutations. Stemmer et al. (U.S. Patent No.  
10 5,514,568) employed overlapping primers with class IIS enzymes to amplify a plasmid and to introduce specific mutations into DNA leaving all other positions unaltered.

There remains a need for the ordering and assembly of complex genes to overcome the problems associated with sequential sub-cloning such as multiple purification steps, the potential for sample loss, and the like. Moreover there is a need for eliminating the  
15 use of prokaryotic hosts and for minimizing or avoiding the risks associated with bacterial contamination resulting from the use of bacteria as intermediaries in the cloning process. Further, there remains a need for efficient methods to assemble large nucleic acid molecules or many-fragmented nucleic acid assemblies with precision.

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#### Brief Description of the Figures

**Fig. 1A.** provides one schematic of six double stranded DNA fragments, each terminus comprising a unique overhanging two-nucleotide sequence complementary to only one other terminus

25 **Fig. 1B.** illustrates a three-piece ligation where 100% of the clones tested contained the predicted fragment order and desired fragment orientation.

**Fig. 2.** illustrates the use of a class IIS restriction endonuclease (as one example, *Bpm1*), restriction endonuclease recognition site and the selection of cohesive overhanging ends.

30 **Fig. 3A.** illustrates an exemplary retrotransposon-derived vector including a murine VL30 LTR (NLV-3) and packaging signal, an internal ribosome entry site (IRES) from encephalomyocarditis virus (EMCV), a gene encoding a green fluorescent protein (GFP), additional internal VL30 sequences (solid bar), SV40 early region promoter and Tn5

aminoglycosidase phosphotransferase (neo) gene, PBR322 plasmid origin of replication and a plus-strand primer binding site (VL30). An exemplary vector sequence is provided as VLBPGN (SEQ ID NO:1). Fig 3B is an illustration of an LTR with the insertion of a U3 (transcriptional promoter)region rescued by reverse transcriptase-polymerase chain reaction (RT-PCR). The promoter is amplified from the RNA of a cell expressing the VL30 U3 region. Complementary overhanging ends are created using class IIS restriction endonuclease digestion sites within the LTR and within the promoter. Fig. 3C provides the linear structure of a VL30 RNA transcript from a mouse cell with a U3 region near the 3'-terminus of the RNA molecule. PCR primers include a class IIS enzyme recognition site to amplify the U3 region from the RNA resulting in a double stranded DNA molecule. Cleavage with a class IIS enzyme (here *BpmI*), results in a double-stranded DNA molecule with end complementary to a site in the vector of Fig. 3A.

Fig. 4A. is a schematic illustrating steps for assembling a combinatorial library of *cis*- or *trans*-acting nucleic acid sequences for assembly and screening, useful for the rescue of biologically active species. Fig. 4b is a diagram of a U3 (transcriptional enhancer and promoter region of an LTR illustrating several sub-divisions of the transcriptional control region, including a distal enhancer region, an enhancer repeat region, a medial promoter and a proximal promoter. These regions have been described for other vectors in Hodgson et al. (1996. "Construction, Transmission and Expression of Synthetic VL30 Vectors" in Hodgson ed. *Retro-vectors for Human Gene Therapy*. RG Landes Company, Austin TX). Segments of these regions are amplified using primers for highly conserved sequences. Highly conserved sequences are determine based on a comparison of known VL30 sequences such as provided in Fig. 4.2 of Hodgson, 1996, *infra*). The parts are joined by annealing and ligation to provide an ordered assembly. Each construct is an allele or a representative of allelic variation in the combinatorial library.

Fig. 5 discloses two transcriptional promoters that have been rescued from mouse VL30 RNA sequences isolated from a mouse T-helper cell library. These promoters were assembled into a vector and introduced into retroviral helper cells and packaged into recombinant retrovirus for introduction into human T-cells. After transduction to human T cells, a  $\beta$ -galactosidase reporter gene was expressed from the T cell-derived promoters.

Fig. 6 discloses 10 biologically active mouse VL30 promoters obtained from mouse liver RNA. These promoters were introduced into the vector of SEQ ID NO:1. The vectors

were introduced into retroviral helper cells and then packaged into retrovirus where they were introduced into human liver cells. The cells expressed the green fluorescent protein.

**Fig. 7** illustrates a similarity plot of nucleotide sequences found in VL30 U3 regions.

**Fig. 8** illustrates a retro-vector comprising six double-stranded DNA fragments that  
5 were self-assembled into a circular structure using unique overlapping termini created using class IIS restriction endonucleases. Three templates and twelve primers were used in conjunction with three class IIS enzymes to make the six fragments that were ligated in a single step. The vector was efficiently self-assembled and was effectively transmitted by both DNA transfection as well as by retroviral transduction of the self-assembled DNA,  
10 without molecular cloning through a prokaryotic host (see Example 2).

#### BRIEF SUMMARY OF THE INVENTION

The invention described herein provides seamless, directional, ordered construction of complex DNA molecules, vectors and libraries. More particularly, it enables  
15 gene constructs to be assembled with greater efficiency and precision, and it enables multiple gene fragments to be assembled in the correct order and orientation without disturbing the internal structure of the gene. The method utilizes *in vitro* assembly of nucleic acid fragments and relies upon the unusual ability of certain enzymes to digest nucleic acid molecules at pre-determined sites without disrupting the structure of the gene. It is especially  
20 useful for the construction of genetic vectors for gene therapy or genetic engineering of cells and organisms. A particular application of the invention is in combinatorial, or evolutionary genetics, where it enables a large number of non-random, self-assembled constructs to be screened simultaneously for function.

In a preferred embodiment of this invention, the invention relates to a method  
25 method for assembling a gene or gene vector comprising the steps of: a) designing at least 6 primers to produce to amplify at least three fragments in at least three separate polymerase chain reactions wherein each primer comprises at least one predetermined restriction endonuclease recognition site that recognizes a restriction endonuclease that cleaves at a distance from the recognition site, a sequence complementary to a template nucleic acid for  
30 amplification, and bases positioned at the restriction endonuclease cleavage site that are selected to be complementary to only one other overhanging created from enzymatic cleavage of the fragments; b) combining the primers with template nucleic acid and performing the

polymerase chain reaction to produce multiple copies of an amplified template fragment incorporating the restriction endonuclease recognition site; c) digesting the amplified template fragments with one or more restriction endonucleases that recognize the restriction endonuclease recognition site of the primers to create overhanging termini wherein each 5 overhanging termini is complementary to only one other overhanging termini on another fragment; and d) combining the amplified and digested template fragments in a ligation reaction to produce a directionally ordered gene, nucleic acid fragment or gene vector.

In a preferred aspect of this embodiment, the restriction endonuclease is at least one class IIS restriction endonuclease and preferably, the class IIS restriction 10 endonuclease is selected from the group consisting of: *AlwI*, *Alw26I*, *BbsI*, *BbvI*, *BbvII*, *BpmI*, *BsmAI*, *BsmI*, *BsmBI*, *BspMI*, *BsrI*, *BsrDI*, *Eco57I*, *EarI*, *FokI*, *GsuI*, *HgaI*, *HphI*, *MboII*, *MnlII*, *PleI*, *SapI*, *SfaNI*, *TaqII*, *Tth111II*. Still more preferably, class II restriction 15 endonuclease recognition sites (to be distinguished from class IIS restriction endonuclease recognition sites), linkers, or adapters are not used to create the gene or gene vector. In one embodiment, the product of the ligation reaction is introduced into prokaryotic or eukaryotic cells. Preferably, at least one template nucleic acid sequence is chosen from the group 20 consisting of : transcriptional regulatory sequences; genetic vectors; introns and/or exons; viral encapsidation sequences; integration signals intended for introducing nucleic acid molecules into other nucleic acid molecules; retrotransposon(s); VL30 elements; or multiple allelic forms of a sequence.

In another preferred aspect of this embodiment, the method is used to generate combinatorial libraries of a target sequence. Preferably, the target sequence is part or all of a gene. In one embodiment, the gene encodes a protein. In one embodiment, the primers amplify allelic variants of part or all of a gene.

25 In still another preferred aspect of this embodiment, the product of the ligation reaction is passed between eukaryotic cells using a virus particle, by cell fusion, or by transfection. Preferably the product of the ligation reaction is not introduced into prokaryotic cells. Moreover, the method further comprises combining at least one screening or selection step to select the products of the ligation reaction. In one embodiment, the product of the 30 ligation reaction is mutated during passage in cells in order to generate genetic diversity and preferably the product of the ligation reaction is mutated by homologous recombination during passage in cells.

In another aspect of this embodiment, the method is used to isolate and identify regulatory sequences from a cell. In another aspect of this embodiment, cells containing the product of the ligation reaction are selected for enhanced biological activity. Preferably, the cells containing the product of the ligation reaction are selected for tissue-specific, hormone-specific or developmental-specific gene expression. Also preferably, the ligation reaction is a circularized gene vector.

In another embodiment of this invention, the invention relates to a nucleic acid primer having a 5' and a 3' end to amplify a nucleic acid fragment for the ligation of at least two fragments comprising: a restriction endonuclease recognition site that recognizes a restriction endonuclease, wherein the restriction endonuclease cleaves at a distance from the recognition site and creates overhanging termini; a sequence complementary to a template sequence to be amplified to produce the nucleic acid fragment; at least two nucleic acid bases positioned at the restriction endonuclease cleavage site and that form an overhanging terminus after cleavage by the restriction endonuclease, wherein the at least two nucleic acid bases are selected to be complementary to only one other overhanging terminus on another fragment of the ligation; and an affinity handle on the 5' end of the primer. Preferably the primer further comprises an anchor to provide stability to the restriction enzyme at the restriction enzyme recognition site.

In yet another embodiment of this invention, the invention relates to a method for isolating and identifying promoters comprising the steps of: a) obtaining a vector comprising at least a portion of a promoter region from a retrovirus transposon LTR and having two non-complementary overhanging termini; b) designing at least two PCR primers to amplify at least one region of a retrovirus transposon LTR from template nucleic acid to produce at least one nucleic acid fragment wherein each primer comprises at least one predetermined restriction endonuclease recognition site that recognizes a restriction endonuclease that cleaves at a distance from the recognition site, a sequence complementary to a template sequence from a retrovirus transposon, and bases positioned at the restriction endonuclease cleavage site that are selected to be complementary to only one other overhanging terminus of the vector wherein the restriction endonuclease cleavage site is created from enzymatic cleavage of the fragments; b) combining the primers with template nucleic acid and performing a polymerase chain reaction to produce multiple copies of an amplified template fragment incorporating the restriction endonuclease recognition site; c)

digesting the amplified template fragments with one or more restriction endonuclease that recognize the restriction endonuclease recognition site of the primer to create overhanging termini; and combining the amplified and digested template fragment in a ligation reaction with the vector to produce a gene vector with an intact LTR sequence. In one embodiment of  
5 this aspect of the invention, the template nucleic acid is DNA or RNA. In another embodiment of this aspect of the invention, the method further comprises the step of sequencing the insert to identify the promoter sequence. In one embodiment promoter sequences of SEQ ID NOS:1-13 identified using the methods of claim.

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#### Detailed Description of the Invention

In one embodiment of this invention, the invention relates to the seamless, oriented self-assembly of at least three DNA fragments having overlapping unique cohesive ends generated by the enzymatic cleavage of at least one restriction endonuclease that is capable of cleaving at a site distant to the restriction enzyme recognition site. Preferably the  
15 restriction endonucleases employed in this invention are class IIS restriction endonucleases. These enzymes recognize a predetermined group of nucleotides and cleave at a distance characteristic of the particular endonuclease from the recognition site. The term "unique cohesive ends" is used herein to refer to the notion that the cleavage site for the endonucleases of this invention can be manipulated to produce overhanging ends with unique  
20 termini selected by the investigator. The term "complementary" as used herein in reference to the overhanging ends of the fragments of this invention refers to standard complementarity recognized in the field of molecular biology. For example, the nucleotides sequence 5'-TAG-  
3' is said to be complementary to the nucleotide sequence 5'-CTA-3'. The term "PCR" is used generally to refer to the polymerase chain reaction and its variations, including RT-PCR  
25 as well as other gene amplification techniques employing primers.

In a first step for practicing one embodiment of this invention, a series of at least three overlapping fragments are created through the selection and creation of primers incorporating at least one class IIS restriction enzyme recognition sequence. The oligonucleotide primers of this invention are designed to amplify one or more nucleic acid  
30 fragments and comprise a sequence complementary to a target sequence for gene amplification, a recognition sequence for a restriction endonuclease that cleaves DNA at a distance from the recognition sequence (such as a class IIS restriction enzyme) and bases

positioned at the restriction endonuclease cleavage site that are preferably unique and complementary to only one other overhanging termini in the annealing/ligation reaction that generates the complex nucleic acid molecules. Optionally, the primers of this invention can include an "affinity handle for cleanup" at the 5' end. These sequences can be of any length, 5 preferably at least about 6 bp and the sequences extend the primer in the 5' direction from the restriction enzyme recognition site. This extra length gives many enzymes greater stability and improved activity. In addition, the sequence can be used for recognition and removal of the ends of the primers (either undigested fragments or digested ends of primers) using complementary nucleotide sequences bound to a solid support (such as cellulose, 10 nitrocellulose or silica). Incubation with, or passage over a column or support containing the complementary sequences can be used to remove the tags by allowing them to anneal or hybridize. The nucleic acid can then be eluted from the column. Adapters can also be used in this invention. For purposes of this invention, adapters refer to double stranded fragments containing an enzyme recognition site, according to this invention. The adapters are ligated 15 to double stranded DNA molecules, creating a fragment analogous to a PCR fragment with similar sites derived from a primer. The primers or adapters can be prepared using a number of methods for synthesizing oligonucleotides known in the art. For example instruments for producing oligonucleotides are available from Applied Biosystems, Inc., Foster City, CA.

In one example, for the design of an oligonucleotide primer for use in this 20 invention, the particular complementary bases that will form the site for hybridization of the primer to template (i.e., target DNA or RNA) are selected. A restriction endonuclease recognition site is selected followed by a number of nucleotides to be positioned between the recognition site and the cleavage site. The nucleotides of the cleavage site are selected to include overhanging regions formed from the restriction endonuclease cleavage that are 25 complementary to the overhanging regions of an adjacent fragment in the annealing/ligation reaction.

The length of the primer used in this invention can vary, but preferably the 30 primer length is up to about 80 bases and preferably up to about 50 bases. In addition the primers are preferably at least about 15 bases in length and preferably at least about 25 bases in length. The 5' region of the primer contains preferably at least about 6, preferably at least about 10 and still more preferably at least about 16-18 bases that are not complementary to the template DNA or RNA. Further, the primer incorporates a restriction endonuclease

recognition site preferably 5' to the region of complementarity and a restriction endonuclease digestion site preferably 5' to the region of complementarity or within the region of complementarity. There are a variety of restriction endonucleases that cleave at a distance from the restriction endonuclease recognition site of a DNA strand and a variety of enzymes 5 that are commercially available from New England Biolabs are provided in Table 1.

**Table 1. Restriction endonucleases useful in the construction of self-assembling genes**

Enzyme:	Site size (bp):	Distance to overlap:	Size of overlap:	Overlap type:
<i>Alw26 I</i>	5	1-5bp	4bp	5'-Overhang
<i>BbsI</i>	6	2-6bp	4bp	5'-overhang
<i>BpmI</i>	6	16-14bp	2bp	3'-overhang
<i>BsmBI</i>	6	1-5bp	4bp	5'-overhang
<i>BspMI</i>	6	4-8bp	4bp	5'-overhang
<i>BsrDI</i>	6	0-2bp	2bp	3'-overhang
<i>Eco571</i>	6	16-14bp	2bp	3'-overhang
<i>FokI</i>	5	9-13bp	4bp	5'-overhang
<i>Hgal</i>	5	5-10bp	5bp	5'-overhang
<i>HphI</i>	5	8-7bp	1bp	3'-overhang
<i>MnlI</i>	5	7-6bp	1bp	3'-overhang
<i>PstI</i>	5	4-5bp	1bp	5'-overhang
<i>SapI</i>	7	1-4bp	3bp	5'-overhang
<i>SfaNI</i>	5	5-9bp	4bp	5'-overhang

10 In addition to the enzymes provided in Table 1, other restriction endonucleases that cleave at a distance from their restriction endonuclease recognition site include, but are not limited to, *AlwI*, *BbsI*, *BbvI*, *BbvII*, *BsmAI*, *BsmI*, *BsrI*, *EarI*, *GsuI*, *MboII*, *TaqII*, *Tth111II* and their respective isoschizomers. These and other enzymes are known in the art and many are available from other manufacturers. The primers can be prepared to produce 15 either 5'-overlapping ends or 3'-overlapping ends, as long as they are both are either 5'-overlapping ends or 3'-overlapping ends and are complementary to one other set of overlapping ends.

In the case of *BpmI* (see Example 1), the enzyme digests asymmetrically, 14-16 bp from the 3'-nucleotide of the recognition site. The resulting cleavage has a 3'-overhanging end of 2 bp. A second primer is then designed with a complementary 20

overhanging end, and it is used to generate the adjoining fragment terminus. At the opposite ends of the two fragments that are to be joined, similar complementary, overhanging ends are designed.

- The oligonucleotides are then combined with template nucleic acid (either
- 5 DNA or RNA, e.g., such as for reverse transcriptase polymerase chain reaction (RT-PCR)) containing bases complementary to at least a 3' portion of the primers (also referred to herein as "templates"). In one embodiment, the fragments are gene-amplified by PCR, RT-PCR or another gene amplification process using established PCR protocols such as those provided with PCR amplification kits, including those available from Perkin-Elmer Corp. (Emeryville,
- 10 California). Preferably, the PCR products are analyzed by electrophoresis on a gel, such as an agarose gel and still more preferably the fragments of the predicted size are purified free of excess primers and small byproducts (such as by purification through a small column, such as a Qiagen™ column (Qiagen, Valencia, CA)). Following amplification or purification, the fragments are digested with the restriction endonuclease recognizing the restriction
- 15 endonuclease recognition site in the primers. The digested fragments are then purified from the digested ends of the primers, preferably by preparative agarose gel electrophoresis. The fragments are combined, annealed and are ligated using standard hybridization and ligation conditions known for cloning (see Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, 1994).

- 20 Fig. 1A illustrates an example of a self-assembling gene construct (SEQ ID NO:1) comprising six fragments, each having unique overhanging dinucleotide ends. In this example, the ends of the fragments prepared by the methods of this invention are constructed using primers that include *Bpm*I restriction endonuclease recognition sites. It will be understood by those of ordinary skill in the art that one or more other restriction
- 25 endonucleases (such as those of Table 1) could similarly be used for the self-assembling product of Fig. 1A. In a preferred embodiment, the primers were created as described above and preferably the 3'ends of the primers are non-palindromic (i.e., non self-complementary) to prevent self-annealing of such fragments. Each fragment in this example preferably joins to only one other dinucleotide overhang in the annealing/ligation mixture, assuring ligation only
- 30 to the intended fragment partner. An advantage of this strategy is that the formation of concatamers or multimers is minimal. The restriction endonuclease site is removed by

digestion with the restriction endonuclease, leaving the junction free of the extra DNA sequences associated with the site.

Using a single restriction endonuclease with a dinucleotide overhang (for example, using the enzyme *BpmI*) up to six pieces of genetic material can be joined together 5 in a linear or circular form (such as a vector) without the need to perform sub-cloning procedures or detailed analysis of individual products because six unique combinations of dinucleotide overhangs create a directional clone with extremely high fidelity. With enzymes digesting single-base overlaps, only two fragments can be joined with positional and directional precision. With enzymes digesting three-base overlaps,  $4^3/2$ , or 32 fragments can 10 be so joined in the correct order and orientation. Therefore, this invention also relates to the use of restriction endonuclease recognition sites that facilitate cleavage by restriction endonucleases with three-base overlaps and self-assembly gene constructs including 32 fragments. Alternatively, a combination of restriction endonuclease recognition sites for use with a combination of restriction enzymes that create two-base or three-base overlaps can be 15 used. Each enzyme has its characteristic limits to self-assembly imposed by the size of the overlap. For example, there are sixteen dinucleotides, therefore *BpmI* fragments (which have two dinucleotide ends each) are limited to eight for the purpose of self-assembly; therefore in another embodiment of this invention an assembly comprising eight fragments is contemplated. However, four of the sixteen dinucleotides are palindromes. Use of these 20 palindromic dinucleotides can create some infidelity in the annealing/ligation reaction. The enzyme *HgaI* has a five base overlap, and there are 1,024 pentanucleotide combinations, permitting 512 fragments to be ligated together directionally and in order (no palindromes). The fragments to be joined at a particular place are designed to have their cut sites aligned, so 25 that the overlapping region fits together. In some cases, the target sequences will contain natural restriction endonuclease recognition sites for the enzyme that is being used, such as one or more internal *BpmI* sites. These sites have the potential to self-religate during vector or gene construction or they can be bypassed by using a substitute enzyme in the primers (for example, Eco 571 can substitute for *BpmI*). Alternatively, these sites can be removed by site-directed mutagenesis after consideration to the consequences of the mutagenized sequence to 30 the gene or vector.

In addition to class IIS enzymes, class II restriction endonucleases can be used. These enzymes have intrinsic methylation activity that affects the outcome in either a

negative or a positive way, depending on the purpose for which it is used. In a preferred embodiment, the methylation activity of class II enzymes is ablated by mutation or by genetic engineering to convert the enzyme to an effective class IIS enzyme to expand the repertoire of useful enzymes for this invention.

5       In another aspect of this invention, the primer design and target fragment sequence selection can be automated (see Example 5) using a computer to assist in the selection of unique overhanging ends that have complementarity only to the overhanging end of an adjacent fragment.

Therefore, this invention permits high-fidelity annealing and ligation of six or  
10 more fragments with unique overhanging termini complementary to a single other overhanging termini. Any multitude of combinations can be created by combining the type of overhanging termini that can be created. Moreover, if one is willing to sacrifice the fidelity of the reaction, a variety of combinations can be used to anneal a variety of fragment numbers. In these cases, some selection may be necessary, such as size selection of the  
15 resulting fragment based on electrophoretic migration or restriction endonuclease profiling, both methods well known to those of ordinary skill in the art

It is also necessary to have a high per-step efficiency (e.g., each step in the process is performed with an efficiency of at least 80%) to effectively ligate large numbers of fragments without error. Where large numbers of fragments are used, the purity of the  
20 fragments becomes important. This means that for large numbers of fragments, the digested DNA fragments for annealing and ligation should be substantially pure. If undigested fragments, digested ends of primers, degraded or partially degraded molecules are present, they can decrease the purity and affect the fidelity of the product. Therefore, it is particularly desirable to ensure complete digestion of both ends of each fragment and to remove all of the  
25 digested ends from the fragments prior to including the fragments in an annealing and ligation reaction. The use of Qiagen columns for oligonucleotide removal prior to digestion is generally sufficient to permit efficient digestion of the fragments. Agarose gel isolation is desirable after digestion particularly where the product contains some fragments that do not appear to be full length. The use of an analytical gel before and after digestion helps in  
30 determining whether both oligonucleotide tags have been removed. The isolation of fragments from agarose gels preferably avoids the use of ultraviolet light and exposure of the

DNA to ethidium bromide is also preferably avoided. These methods can be avoided by running replicate lanes and staining only a portion of the gel.

The fragments and vector are then digested to yield fully complementary ends, and the fragments are preferably again purified, as described above (such as through a Qiagen 5 column or by gel isolation). The purified fragments are ligated together in a test tube, under standard conditions, such as using bacteriophage T4 DNA ligase and ATP. Preferred ligations include at least 20 $\mu$ g/ml total DNA concentration in the ligation mix to favor intermolecular interactions, and an equimolar ratio of fragments to be ligated. Where a prokaryotic intermediary is used, the ligated assemblage is transformed into a bacterium, such 10 as an *E. coli* host, and the colonies are: selected with a drug (such as an ampicillin, tetracycline, or kanamycin marker). The colonies can then be selected either by individually selecting colonies or growing a mass culture, such as where a vector library has been created. Restriction enzyme analysis can be used to determine the identity of individual constructs or to assess the validation of the combination of plasmids. The plasmids can then be grown up 15 and used as needed.

In one embodiment of this invention, at least a portion of a vector is used as one of the fragments for the ligation of at least three fragments according to this invention. In one example, where a vector is used as one of the starting fragments, two restriction endonuclease recognition sites recognizing an enzyme that cleaves at a distance from the 20 recognition site, such as at least one *BpmI* site, can also be introduced into the vector. This permits the vector to be digested with the restriction endonuclease to produce a product having ends complementary to two ends of the insert DNA fragments. The vector can be made by amplifying a plasmid or portion thereof using the primers of this invention. Thus, the vector can also be constructed to include a variety of restriction endonuclease recognition 25 sites using a variety of restriction endonucleases, including a variety of class II restriction endonucleases. In some cases, the target fragments for amplification will contain natural restriction endonuclease recognition sites for the enzyme that is being used for the self-assembly, such as for example, a fragment that includes one or more internal *BpmI* sites. Care should be taken either to utilize the complementarity of the naturally occurring site to 30 reform the fragment as it originally existed or to eliminate the restriction endonuclease recognition site using, for example, site-directed mutagenesis. Preferably, the restriction endonuclease recognition site is substituted for a different enzyme (in the case of *BpmI*,

substituting *Eco*57I or *Bsr*DI) that has an equivalent structure at its ends. Two or more fragments of insert or two or more fragments of vector with at least one insert are amplified using primers according to this invention.

The exemplary enzyme, *Bpm*I digests DNA 14-16 base pairs (bp) from the 3'- nucleotide of the recognition sequence (RS). Thus, by placing the RS exactly 14-16 bp from the desired dinucleotide cut site, the practitioner tags the dinucleotide for ligation with another dinucleotide that is exactly complementary to it. Such a complementary dinucleotide can be inserted by using the same enzyme and RS to make another fragment which fits the first exactly, as illustrated in Fig. 1. Because there are sixteen possible dinucleotide combinations (including twelve combinations that do not have palindromic ends), it is possible to create up to six fragments with unique dinucleotides, and it is also possible to join them all together in a predetermined order and orientation (Fig 1A). In addition, the palindromic sequences (such as AT, CG, TA, and GC) could also be used, although inefficiency and incorrect ligation will result from the self-complimentarity of these sequences. It is furthermore possible and desirable to have three or more fragments joined in this way, such that the construct is circular as in Fig. 1, comprising a vector that may be grown in a bacterial and/or eukaryotic host cell. If the genetic construct is to be used as a vector, the vector should be designed to include a proper origin of replication to enable it to replicate in a particular cell. For example, a prokaryotic origin of replication such as a coliform plasmid origin of replication enables circular DNAs to be propagated in *E. coli* host cells. It is desirable to have at least one selectable marker, such as a neomycin marker that enables recovery of the clone through a selection process. It is also desirable, but not essential, to have two or more selectable genetic elements, to permit dual selection. For example, if one of the fragments contains a prokaryotic plasmid origin of replication, and another fragment contains a selectable marker, then the two fragments are both selectable, since the construct will grow in prokaryotic cells in the presence of a selection drug (such as ampicillin) only when both fragments are present. Drug selection can be combined with the methods of directed self-assembly to assure a high percentage of correct products. Because of the unique complementarity of the fragments, each contributes a selectable element that leads to recovery of a high percentage of correct products.

For prokaryotic vector construction, at least one fragment should contain a prokaryotic origin of replication and one fragment should contain a drug resistance marker

gene. However, an advantage of the methods of this invention is that the construct can be introduced directly into eukaryotic cells. Here no plasmid origin of replication is necessary and no prokaryotic selectable marker or other prokaryotic nucleic acid sequence is necessary. In cases where the vector is subject to regulatory approval or where optimal gene function is necessary, it may be undesirable to include prokaryotic sequences, such as extraneous plasmids or expressed prokaryotic fragments particularly if the sequences contain immunostimulatory sites that can lead to activation of the intracellular immune system and inactivation of a gene product (see Krieg et al., *J. Lab. Clin. Med.*, 128:128-133, 1996) or to avoid risks of endotoxin contamination. Moreover, the use of self-assembled product, according to the methods of this invention saves labor and time involved in the screening process.

Thus, in a preferred embodiment of the invention, the nucleic acid fragments are self-assembled *in vitro*, and are transferred directly into eukaryotic cells, by transfection, injection, or other methods known in the art. In one embodiment the cells receiving the assembled product of this invention are helper cells for recombinant virus assembly (including, but not limited to retroviral helper cells for retroviral or retrotransposon vectors, adenovirus helper cells for adenovirus vectors or herpes simplex virus helper cells for herpes simplex vectors). Alternatively, the assembled product can be introduced into cells along with a helper virus or the assembled product can be introduced into target cells for direct expression. The assembled product can be a vector, a minichromosome vector, a portion of a chromosome, or the like. In the preferred case of a retroviral vector, the genes are first transfected into a first helper cell line (such as ecotropic helper cells, GP+E86 (Markowitz et al. *J. Virol.* 862:1120-1124, 1988). The retrovirus-containing supernatant from these cells is then filtered (0.45mm Nalgene filters) preferably 48-72 hours after transfection and the filtrate is transferred to a second complementation retroviral helper cell line (such as PA317 retroviral helper cells, Miller et al., *Mol. Cell. Biol.* 6:2895-2902, 1986). After an additional 48 h, the second helper cell line is selected with the marker drug (such as the drug G418 for the selectable neomycin (neo) marker gene), until only drug-resistant cells remain. These cells contain stably integrated vectors that can be used to repeatedly transduce human cells. Advantageously, in the case of adenovirus vectors or other large eukaryotic -derived vectors including eukaryotic virus-derived vectors, it may be impossible to propagate them in prokaryotic hosts. The gene self-assembly method of the instant invention provides an

alternative to *in vitro* recombination method of gene construction by permitting large constructs to be constructed.

One advantage of introducing the assembled product of this invention into a helper cell line to produce recombinant virus for the introduction of a gene or nucleic acid 5 complex into a cell is that the assembled product will be auto-selected by the cells during the packaging process. Therefore, even where the overhanging termini have palindromic sequences, where there is more than one (but preferably less than four) unique complementary matches for a particular overhanging termini, or where concatamers have formed, only the correct or functional assembled products are expressed, transmitted, and 10 assembled into virus. When the virus is then introduced into cells, the use of a reporter gene or another selectable marker provides yet a second layer of security for the selection of cells containing a properly assembled construct. For example, where a retrovirus helper cell line is used to produce a recombinant retrovirus containing the product of this invention (for retrovirus, RNA transcribed from the DNA product of the invention becomes packaged into 15 the virus particle), a retrovirus-derived vector is transcribed as RNA and transmitted by packaging the RNA in a retrovirus particle. In order to be properly transmitted as a virus, the construct must be: 1) transcribed as RNA in a vector producer cell; 2) packaged into viral particles; 3) reverse transcribed into double-stranded DNA (in the recipient cell); and 4) integrated into the host chromosome. Each of these steps requires specific *cis*-acting 20 sequences that must be correctly positioned within the vector. Thus, passage via retrovirus (or by other virus) is a means of auto-selection for the essential sequences.

In one application of the methods of this invention, the methods are used to rescue expressed sequences from RNA, or genomic sequences from cell DNA without disrupting the promoter sequences. Cellular transcriptional promoters are typically difficult 25 to identify and isolate because they are generally not included in the RNA molecule and often extend over a considerable distance in a chromosome. One application of this invention relates to a promoter rescue technique that permits the entire promoter, or a fragment of a promoter to be isolated and cloned directly into an expression vector without disruption of the flanking sequences. Promoter rescue techniques are known and include WO 94/20608 to 30 Hodgson.

In a preferred embodiment of the invention, transcriptional promoters are cloned in a transcriptionally active manner for the selection and identification of new and/or

of tissue or cell-specific promoters enabling them to be used, selected, or screened for activity directly. For example, Fig. 3 illustrates one example of the formation of a vector for the incorporation of promoter sequences and the ultimate identification of those sequences using an exemplary plasmid VLBPGN (SEQ ID NO:1) as provided in Example 3, with *Bpm1* sites located within the locus of a retrotransposon (VL30) long terminal repeat (LTR). These methods preserve the structure and functionality of transcription factor response elements. The characteristic secondary structure of the LTR RNA remains very similar to the original LTR from which the promoter was rescued, thus preserving the important features of the original RNA/DNA molecule. Those of ordinary skill in the art will recognize that any of a variety of primers can be used with a variety of vectors and that the constructs of Figs 2 and 3 are exemplary and not limiting.

Fig. 2 illustrates the primers used to amplify the promoter insert (identified at a and c in Fig. 2), and the insert region of the LTR (boxed), both of which can be digested at the same nucleotide position with *Bpm1*, to ensure a proper and seamless fit. In this example, after digestion of the vector, the two *Bpm1* sites leave non-complementary ends (a 3'-CC overhang on one end, and a 3'-GC overhang on the other). Thus, the ends will not efficiently anneal or ligate to one another. However, the complementary termini of the insert serves as linkage, enabling the plasmid to be completed by ligation.

In the example illustrated in Fig. 2, the terminus on the 3'-side (GC) is palindromic. Palindromic termini are self-complementary and can therefore ligate to themselves or to an identical terminus facing the opposite way (forming concatamers in the opposite direction). Despite the presence of palindromic termini and despite the potential for reduced fidelity in the self-assembling process, a large percentage of clones obtained by inserting promoter sequences into VLBPGN were assembled correctly (20/23). These levels are reduced somewhat when three or more fragments are combined for self-assembly, according to this invention and preferably, the use of palindromic termini are avoided when even numbers of nucleotides are exposed as overhanging termini because with even numbers of nucleotides there is an axis of symmetry. As noted above, where five base overhangs are used there are 1024 possible combinations of five nucleotides [(4)<sup>5</sup>], yet none of them is palindromic.

The vector of Fig. 3 is an example of a particular type of vector that is known as a retrotransposon vector. Retrotransposon vectors are described and reviewed in Hodgson

et al., 1996 *Retro-Vectors for Human Gene Therapy*. RG Landes Company, Austin TX, chapter 5 and see US Patent 5,354,674 to Hodgson. This type of vector is derived from a mouse cellular retro-transposon element that has no essential viral or cellular genes, and that has little sequence similarity to a retrovirus. However, this RNA (known as VL30 [virus-like, 5 30S]) has all the necessary *cis*-acting structural elements (such as LTRs and primer binding sites) required for efficient transmission by a type C murine or primate retrovirus. Thus, it is a parasite transmitted by retroviruses that is also expressed as a cellular RNA in most mouse cells and tissues. This RNA becomes packaged into retroviral particles when the mouse cells become infected by retrovirus. The retrovirus then transmits the VL30 (or a VL30 vector) to 10 the next infected cell (which can be a human cell). The RNA is then reverse transcribed and integrated into the DNA of the host cell.

Some advantages of VL30 vectors (over retrovirus-derived vectors) are: 1) lack of viral genes and other sequence homology that could lead to replication competent retrovirus (RCR); 2) ability to be expressed long-term *in vivo*; 3) a variety of LTR 15 transcriptional promoters that can be expressed in various tissues and under the influence of various hormones and other stimuli; and 4) the ability to express genes in a number of cell types that are targets of gene therapy. An additional advantage is that VL30 parts can be switched with those of classical retrovirus-derived vectors. For example, the LTR or packaging signal of VL30 can be used in place of the equivalent retroviral signal. The ability 20 to make mixed, or chimeric retro-vectors is a special application of gene self assembly technology.

Using a specific primer set, such as that shown in Fig. 2, or others, as taught in this invention, it is possible to amplify the U3 sequences expressed in the RNA of many different types of mouse cells. This is done using standard RNA isolation methods (Ausubel 25 et al., *supra*), coupled with extensive digestion with ribonuclease-free deoxyribonuclease, to eliminate residual DNA. Thus, to obtain a promoter that is expressed in the liver, one isolates RNA from liver and uses an RT-PCR procedure, such as those known in the art, with the primers to amplify the desired promoters. Fig. 6 illustrates liver RNA-derived promoters obtained using the methods of this invention. However, the promoters can also be derived by 30 conventional PCR from cDNA libraries (Fig. 5 illustrates T cell-derived promoters that were obtained in this manner). It is also possible to use the well-known hormonal and pharmacological inducibility of VL30 LTRs to find LTRs that are responsive to peptides,

hormones, and cytokines (for a table and description of VL30 pharmacologic responses (see Hodgson et al., 1996 *Retro-Vectors for Human Gene Therapy*. RG Landes Company, Austin TX, chapter 4, and Fig. 4.2). Examples of substances inducing various VL30 promoters to high levels include: epidermal growth factor, basic fibroblast growth factor, insulin, 5 erythropoietin, glucocorticoid hormones, activators of cyclic 3'-5'AMP, and others. To rescue promoters with pharmacological responsiveness, cells or animals stimulated with the desired pharmacological agent are subjected to the RT-PCR procedure and the resulting U3 regions are cloned into a vector, (such as the exemplary VLBPGN) and are tested for inducibility. Standard RNA blotting procedures can be used before isolating VL30 10 promoters, to determine whether a particular drug or hormone causes induction of VL30 RNA expression in a particular mouse cell or tissue. After the promoter has been rescued, the vector is transmitted via retrovirus to the target cell (possibly a human equivalent of the mouse cell from which the promoter was rescued). After selection with the drug G418 (400-700 µg/ml, for 7-10 days) to select against cells not containing the vector, the target cell 15 population is challenged with the pharmacological agent of choice. Reporter gene expression (in the example, GFP) or RNA expression, as determined by RNA blotting, can be used as an assay of gene inducibility by the agent (for exemplary gene expression methods, see Chakraborty et al., *Biochem. Biophys Res. Commun.* 209:677-683, 1995).

Using any specific primer set designed for use with VL30 retro-elements and 20 using total cellular RNA from a particular mouse cell type as a template for RT-PCR, (using commercially available kits and methods therein) candidate promoter elements can be amplified. This method is useful for the identification of mouse-derived promoters and in particular the method is useful for the identification of cell-type specific or tissue-specific promoters from a mouse and for the selection of these promoters and the identification of 25 tissue-specific or cell-specific promoters that function in human cells. Thus, these types of vectors and the methods for using these vectors permits the identification of promoters to permit controlled transcription of a foreign gene. The promoters, originally obtained from the mouse, can be used to effect tissue-specific or cell-specific expression in a human or animal liver cell such as a hepatocyte, or in a human blood cell such as a T-helper cell or in an 30 erythrocyte (red blood cell). Methods are disclosed in Example 2 for the screening and selection of the promoters from a library of amplified promoter sequences. Other methods are well known to those of ordinary skill in the art. The specificity of the selected promoter

can be assessed, for example, by introducing a selectable marker under the control of the test promoter in question and introducing this construct into various cells to assess the ability of the promoter to selectively regulate expression.

The amplified fragments represent U3 promoter regions from any RNA species expressed in the originating cells and their abundance will be in approximate proportion to the number of expressed copies of RNA in the original mixture. Example 3 illustrates one example using a mouse T-helper cell cDNA library to produce amplified fragments representing U3 regions expressed in T cells. The vectors were efficiently expressed as RNA and protein in PA317 helper cells, and were transmitted by retrovirus into human T-helper cells, where they were integrated and expressed as protein in the form of a  $\beta$ -galactosidase reporter gene, as visualized by X-gal staining. The products of this experiment are provided in Fig. 5 and as SEQ ID NOS: 2 and 3 from T-helper RNA. The products of another experiment are shown in Fig. 6 as SEQ ID NOS: 4-13 from mouse liver RNA (by RT-PCR).

Examination of the different U3 sequences isolated from T cells and from liver revealed several things. First, the T cell U3 sequences were related to each other, as were the liver sequences. However, the two types of U3 sequences were quite different between the two sources (T-cell, Figure 5 and liver, Figure 6). Specifically, the liver sequences (Figure 6) appeared to be a closely related group, differing mostly by single point mutations, some of which may affect transcription factor binding sites. Some of the polymorphic sites included: a phorbol ester response element (VLTRE); a Rel/NF $\kappa$ b binding region, and a possible glucocorticoid response element (GRE). Some of these polymorphisms are illustrated in Fig. 6. The T cell-derived sequences (Fig. 5, SEQ ID NO:2 and 3), on the other hand, differed significantly in length, with SEQ ID NO:3 missing more than 120 bases (compared with SEQ ID NO:2) including putative binding sites for retinoids (RAR/RXR) and several elements contained within the enhancer repeat region (including a cAMP response element (VLCRE, or CREB/jun binding site), and putative serum response element (SRE, CARG, and NF1/IL6). SEQ ID NO:3 represented one out of five clones sequenced, while SEQ ID NO:2 represented four out of five. Possible sites of interactions between transcription factors and DNA can be observed by comparing the experimentally derived U3 sequences with those in Hodgson et al. ,(Retro-Vectors for Human Gene Therapy, 1996 Fig. 4.2 *supra*). In addition

to the deleted sequences of SEQ ID NO:2, there are a number of single base differences within the conserved regions of the two T cell-derived sequences.

Advantageously, a number of new VL30 promoter sequences (SEQ ID NOS: 2-13, *supra*) were identified using these methods despite the fact that VL30 RNA comprises only about 0.3% of cell mRNA represented in a cDNA library. Moreover, in each case, the cloned insert was isolated without the need to use linkers, adapters, or multiple cloning sequences such as those that are typically used for other library construction methods. The promoter sequences can be used in the vectors disclosed here to express inserted foreign genes or the promoter sequences can be substituted into other retroviral vectors, such as MoMLV-derived vectors or other VL30-derived vectors. Further, vectors containing the promoter sequences can be propagated in retroviral helper cells, such as PA317 (U.S. Patent 4,861,719 to Miller) or introduced into cells by chemical or physical transfection.

In another application of the methods of this invention, libraries of amplified sequences can be incorporated into vectors using two or more fragments and using the restriction endonucleases cleaving at a distance from their recognition sites. Preferably the vectors are created using six or more fragments and preferably greater than 10 or more fragments. For example, as applied to VL30 promoter sequences, because there are over a hundred VL30 retro-elements in the mouse genome, it is possible to amplify all of the promoter sequences *en masse*, and propagate them *en masse*, enabling screening by serial passage through helper cells (such as the PA317 helper cell line) or by means of a replication competent retrovirus, as illustrated in Examples 3 and 4. Conversely, the promoter region may be broken down into several sub-domains and permutations of each could be combined and screened to enhance the chances of generating a superior construct (Fig. 4B).

As an example of breaking a promoter region down into several sub-domains, Fig. 7 illustrates a similarity plot of nucleotide sequences found in VL30 U3 regions. Plot similarity was performed using the Plot Similarity program (Wisconsin Sequence Analysis Package, release 8.1, Genetics Computer Group, Madison, WI). This program plots the running average of the similarity among the sequences in a multiple sequence alignment. The sequences compared were those found in Fig. 4.2 of Hodgson, 1996, chapter 4 (*infra*). That is, the plot discloses the degree of conservation of VL30 promoter sequences among known VL30 promoters. From the figure, it can be seen that conserved sequences (close to 100% conserved) can be used as primer binding sites to amplify the adjacent sequences by PCR.

An allelic mixture of three fragment sets is then created to make a combinatorial library of promoters that can be positively selected, such as by using retroviral amplification of the active sequences. This, used in combination with the Fig. 4.2 (Hodgson, 1996, chapter 4 *supra*) can be used to determine regions of high similarity. Regions of high similarity within 5 the U3 region can be replaced with one another. Therefore, a library of permutations of these sections can be made by combining allelic pools obtained by amplifying the sequences from individual subsections, followed by ligating the subsections in the correct order using the methods of the instant invention for gene self-assembly. For example, sub-section 1 can include the distal enhancer (from the LTR 5'-end to the site of insert primer 2, see for 10 example the region defined by the insert primers 1 and 2 (SEQ ID NOS 55 and 56 of Example 4). In this way, using a plot similarity (such as Fig. 7), within each sub-section, the primers position fragments within a region of nearly 100% identity. Degenerate primers can also be used in these experiments to account for multiple nucleic acid base combinations along a particular sequence. In each case, the primers preferably are designed to have a 15 melting temperature that is compatible with the RT-PCR conditions being used, and the conditions should be those recommended by the manufacturer (preferably Perkin Elmer Corp., Emeryville, CA). In Example 4, a set of primers is given that can be used to amplify different U3 subsections, together with directions for assembling a combinatorial library.

It will be appreciated by persons of ordinary skill in the art that the methods 20 of the instant invention can thus be used to make allelic libraries of a variety of genes. For example, different allelic portions of a gene can be combined in a predetermined order and orientation to produce combinatorial libraries, without the need for fortuitous restriction sites separating the parts in the original construct, and without perturbing the important sequences joining the parts using the methods of this invention.

In this invention primers are constructed as described above. However, for the 25 generation of allelic libraries or more complex library constructs it may be helpful to include 5' tags into the 5' end of the primer. The purposes of the tag sequence are: 1) to provide extra nucleotides on both sides of the restriction endonuclease recognition sites (for more efficient digestion); and 2) to enable recovery of sequence tags or undigested fragments by means of 30 an affinity reagent (such as silica, magnetic beads, or nitro-cellulose containing the complementary sequences) for purification. The use of an affinity reagent permits the digested ends to be purified away from the digested fragments. Furthermore, if any

undigested ends remain after thorough digestion, the affinity reagent will remove them, further aiding in the purification. In one embodiment, affinity purification of the digested fragments is used in place of gel isolation, eliminating possible damage caused by ultraviolet light as well as possible damage caused by dye (e.g., ethidium bromide) binding to the DNA.

5 It will also be appreciated that a number of other variations to the primer sequences can be employed. For example, as discussed above, the enzyme recognition site for an enzyme that digests outside of its recognition sequence is included in the primer, so that the DNA digest creates an overlapping end that is complementary to one other terminus to which it will be joined. The enzyme recognition site can be moved to any location within  
10 the primer so as to digest the DNA at the exact location desired. The primer can also be programmed with a novel enzyme recognition sequence to add any desired sequences between the two sequences to be joined or to incorporate a linker or adapter if desired. If the sequences to be amplified contain the enzyme recognition site of the primers, it may be necessary to switch to a different enzyme usage. The use of several different enzymes is  
15 possible and has been discussed above. As with other PCR procedures, after the initial primer selections have been made the primers are assessed for their ability to fold back on themselves or to create internal secondary structure. The primers are preferably modified to avoid palindromic sequences or the potential for self folding within a primer. Nucleic acid analytical software (such as the Wisconsin GCG package, Oxford Biomolecular, Oxford, UK)  
20 is available to perform this analysis and aid in the selection of alternative primers.

In addition, as with all PCR processes, it is necessary to determine the melting temperatures ( $T_m$ ), and to adjust the annealing temperature of the PCR reactions to compensate for such temperatures. Finally, it is important to perform a sequence redundancy search, to determine whether the target sequence (the sequence complementary to the primer)  
25 is found more than once in the region to be amplified. If the sequence is repeated, it will be necessary to use a different primer in order to establish the single, correct priming site. Preferably, no more than 6-8 bases of incorrect target complementarity at the 3'-end of the complementary region is used and to allow a difference of at least 10° C between the  $T_m$ s of the correct and the incorrect target. The annealing temperature should always be at least 5°C  
30 lower than the  $T_m$  of the correct target and 5°C above the  $T_m$  of the incorrect target. Again, the necessary software and instructions are readily available from the cited sources (Wisconsin Gene Computer Group and Oxford Biomolecular, *supra*)

Next, a vector is constructed to include the appropriate elements for expression in the desired cell type. For example, the plasmid of Fig. 3A can be used for the creation of a promoter library or a vector can be created using a commercially available vector and primers to create a three or more fragment annealing and ligation reaction as provided above.

- 5 Preferably, the inclusion of a dominant negative selectable marker on the vector (e.g., the neomycin phosphotransferase gene, conferring G418 drug resistance) can be used to reduce the likelihood that cells without the vector are being maintained in culture.

Multiple allelic copies of DNA (cell derived or cDNA) can be amplified in separate reactions as a set of potential inserts with each set having its own unique overlap 10 sequence following digestion with a restriction endonuclease, according to this invention. The fragments can then be ligated into an existing vector or in a single reaction of three or more fragments to form a combinatorial collection of potential alleles. For example, if six adjacent regions are amplified from five separate alleles, the number of combinations would be  $5^6$ , or 15,625 potential combinations. The combinations can then be grown *en masse*, and 15 selected *in vitro* or *in vivo*. A variety of screening strategies can be used in this invention and those of ordinary skill in the art will appreciate that the type of screen will match the type of library being generation. Therefore, for the promoter library, introducing members of the library into particular cell types to assess for expression in one or more cell types versus the absence of expression in another cell type is evidence of tissue-specific or cell-specific 20 expression. For screening purposes, the libraries of this invention function like other libraries created through other methods. A variety of screening methods for a variety of libraries have been described in the art. For example, selective screens are reviewed by Hodgson et al. (1996, RG Landes Company, *supra*). Reporter protein production is well known in the art as 25 is dominant selectable marker (e.g. drug) selection and selection by fluorescence activated cell sorting, antibody affinity selection, phage display selection (such as commercially available from Amersham, Milwaukee, WI), and the like can be used without detracting from this invention.

In this way, it is possible to isolate multiple forms of genes, gene fragments or regulatory regions such as transcriptional promoters or packaging signals (for example, in a 30 retro-vector system). The individual constructs may then be tested *in vitro* or *in vivo* to further characterize a particular phenotype.

In one example the method is used to create a library of complementarity determining regions (e.g., allelic variations that give rise to antibody diversity) of antibodies or from receptors, including T-cell receptors, epitopes, antigens, ligands and the like. For example, where a library of T-cell receptors is created, the introduction of a vector designed 5 to create a functioning T-cell receptor can be introduced into T cells or T-cell progenitors and the cells can be tested for their ability to bind to a particular test ligand. The ligand-recognizing cells can then be isolated from the ligand and grown in the presence of cytokines to produce specialized T cell clones. Where a library of antibodies or antibody fragments is created, the antigen reactive portions, for example, can be recombined in a vector containing 10 the remaining portions of an antibody molecule to generate antibodies or antibody fragments in a cell. In other examples, the methods of this invention can be used to create allelic domains of receptor families (such as the steroid receptor super-family); libraries with related regions from peptide hormones; cytochromes P450; or other protein families that have shared domains or sub-sections with similar structures. The methods of the instant invention allow 15 the joining of allelic sub-sections in an ordered fashion. In each case, it will be necessary to design primers, and to keep track of the uniqueness of joining overlaps and the presence of internal restriction sites as described above. While these will be different in each case, here are listed some general guidelines that are incorporated into the method of the instant invention.

20 As discussed above, although described as it relates to promoter libraries, libraries of other nucleic acid sequences can be created using the methods of this invention. These libraries include, introns and/or exons and/or functional domains libraries, libraries of potential alleles for a particular gene sequence, and the like. These sequences can be amplified from cell DNA or RNA using the primers of this invention and incorporated into a 25 variety of vectors. For example, one vector of this invention, VLBPGN, has a portion of LTR removed and can be used to create a variety of libraries following digestion with *Bpm*1.

30 Selected or screened products of the combinatorial library can be used for gene expression, such as the promoters of Figs. 5 and 6. In addition, the exploitation of these sequences for the expression of a variety of genes, the LTR fragment containing the promoter can be joined to one or more functional retroviral packaging signals, internal ribosome entry sites, additional promoters, coding regions, processing sites, and the like.

Advantageously, there are almost no spatial constraints upon the joining of molecules by the method of the instant invention and other methods have not taken advantage of the combination of PCR to isolate genes or gene fragments; enzymes cleaving at a site distant from their restriction endonuclease recognition site to combine three or more

5 fragments with precision; and, the use of unique overlapping non-palindromic termini to ensure fidelity of multi-fragment ligations. This combination permits the artisan to prepare complex gene constructions in one ligation step and does not require sequential sub-cloning into a vector or propagation in a prokaryotic host. Added to this the combination by these methods of fragment pools facilitates recombinatorial genetics.

10 The ability to recombine (in the correct order and direction) and screen a large number of allelic variants (whether as a simple library or as a combinatorial library), resulting in increased abundance (by amplification in the RNA, and subsequently in the DNA) is a special characteristic of this invention. Particular advantages of this system are obtained when the methods of this invention are combined with retrovirus vector technology or other  
15 virus vector technology. For example, the combination provides a form of *in vitro* evolution whereby the passage of the library through virus and through cells selects functioning sequences and increases the abundance of the surviving RNA and DNA molecules.

For example, consider the consequences of screening several different promoters expressing RNA in a donor cell (*i.e.*, a cell producing virus particles), but at  
20 differing levels of RNA abundance. In the following example, the least abundant RNA species is expressed at 0.1 copy of RNA per cell, while six others are expressed at 1 copy, 10 copies, 100 copies 1,000 copies, or 10,000 copies, or 100,000 copies/cell, respectively. After a single passage, the DNA copy number in the recipient cells now reflects the approximate RNA copy number in the donor cells. These numbers are further amplified in the relative abundance of RNA species produced in the recipient cells. Disallowing for factors such as  
25 position effects, transcription factor depletion, etc., (which may be considerable), the same relative ratios of expression would be expected. Taking into consideration position effects, the disparity between abundance caused by changing insertion loci should average out. The most abundant RNA species after two passages is then many orders of magnitude more  
30 abundant than the least abundant.

Species:	RNA	DNA copy	RNA	DNA copy	RNA
	abundance:	no.	abun.	no.	abun.
	P=0	P=1	P=1	P=2	P=2
A	0.1 copy/cell	0.1	0.01	0.01	0.001
B	1	1	1	1	1
C	10	10	100	100	1,000
D	100	100	10,000	10,000	10 <sup>6</sup>
E	1,000	1,000	10 <sup>6</sup>	10 <sup>8</sup>	10 <sup>9</sup>
F	10,000	10,000	10 <sup>8</sup>	10 <sup>8</sup>	10 <sup>12</sup>
G	100,000	100,000	10 <sup>10</sup>	10 <sup>10</sup>	10 <sup>15</sup>

**Table 2.** Enhancement of DNA and RNA copy number as a result of different RNA expression levels, after retroviral passage. P= (no. of passages). Numbers are interpreted as relative ratios within a column.

5

The present invention is able to efficiently create a library of RNA or DNA sequences whether or not they are in low abundance. The kinetics of screening for RNA abundance of a promoter can be appreciated best in the following discussion. For the purposes of this discussion, position effects have been ignored. An equation describing the 10 kinetics of screening for RNA abundance is:

$$(1) R_{\text{rel}\chi} = A_\chi / \sum A_{\chi \infty}$$

The above equation (1) can be stated in plain English: The relative abundance 15 of an RNA species  $\chi$  ( $[R_{\text{rel}\chi}]$ ) within a population of RNA molecules expressed in a single cell or within a population of cells) is equal to the RNA copy number of RNA species  $\chi$  ( $A_\chi$ ) divided by the sum of the RNA copies of all RNA species present, including  $\chi$ .

The relative abundance number of any given species changes as the number of 20 passages change, according to the following approximation:

20

$$(2) R_{\chi py} = D_{\chi p0} R^{p+1}$$

In the simplest of terms, equation two (2) can be expressed as: The abundance 25 of RNA species  $\chi$  after Y passages ( $R_{\chi py}$ ) is equal to the initial abundance of the DNA for species  $\chi$  at passage=0 ( $D_{\chi p0}$ ), multiplied by the RNA abundance/DNA copy, raised to the power of the number of passages plus one. Thus, a typical RNA species that starts out as a

single copy of DNA, after zero passages (*i.e.*, in the donor cell) expresses 10 copies of RNA/cell. After one passage it is amplified at the DNA level to a relative ten copies (the same as the RNA abundance at P=0), and at the RNA level to 100 copies (10 copies per DNA copy). The reason for the amplification is that viral packaging and passage is based upon the 5 number of RNA copies present in the donor cell. These calculations can be used to arrive at approximate abundance determinations for any given passage. The actual results of any given experiment, of course, will be biological rather than physical or mathematical. This means that other variables such as RNA efficiency of transmission and longevity, availability of transcription factors, experimental variation, *etc.* also come into play. The underlying 10 purpose of the approximating equations, however, is to illustrate that RNA is amplified in DNA in proportion to the abundance of the template (RNA) within the cell.

The abundance of mRNA in cells can vary continuously from less than a copy per cell to nearly 100,000 copies/cell in actively transcribing, highly-specialized cells such as reticulocytes, the chicken oviduct, the silk moth silk gland, etc. Therefore, the spectrum of 15 RNA abundance from 0- $10^5$ /cell is within the biological window of interest. For most practical purposes, such as biotechnological expression of genes in specific cells, only the higher end of this abundance range is desired. Therefore, using a viral selection system, as disclosed in this invention, it may be possible to disregard those species with less than a threshold level, such as <0.1 copies per cell. The selection through virus will lead to the 20 recovery of the more abundant species. Furthermore, because the vector is likely to be the only considered sequence, it may be considered as a proportion of the whole of RNAs expressed in the target cell. The situation is more complex when a large number of permutations and combinations is generated, for example by self-assembling thousands or millions of fragments in a predetermined order using the self-assembly technique of the 25 instant invention. Consider the assembly of allelic variants of four promoter subregions: distal enhancer, proximal enhancer, distal promoter and proximal promoter. If 100 varieties of each of the four groups were amplified and combined using the instant process along with a single vector,  $10^8$  resultant combinations could occur. However, a sufficient number of molecules to start out a combinatorial screening program might be a million. The problem 30 can be simplified by considering these in groups as follows:

**Table 3. Grouped abundance of RNA molecules derived from combinations.**

No. of species in group:	RNA abundance:	Total No. RNA molec. at P=0:	RNA at P=1	RNA at P=2	RNA at P=3
9 X 10 <sup>5</sup>	1	9 X 10 <sup>5</sup>	9 X 10 <sup>5</sup>	9 X 10 <sup>5</sup>	9 X 10 <sup>5</sup>
2 X 10 <sup>5</sup>	10	2 X 10 <sup>6</sup>	2 X 10 <sup>7</sup>	2 X 10 <sup>8</sup>	2 X 10 <sup>9</sup>
2 X 10 <sup>4</sup>	1,00	2 X 10 <sup>6</sup>	2 X 10 <sup>8</sup>	2 X 10 <sup>10</sup>	2 X 10 <sup>12</sup>
1 X 10 <sup>3</sup>	1000	1 X 10 <sup>6</sup>	1 X 10 <sup>9</sup>	2 X 10 <sup>12</sup>	2 X 10 <sup>15</sup>
1 X 10 <sup>1</sup>	10,000	1 X 10 <sup>5</sup>	1 X 10 <sup>9</sup>	1 X 10 <sup>13</sup>	1 X 10 <sup>17</sup>
1	100,000	1 X 10 <sup>5</sup>	1 X 10 <sup>10</sup>	1 X 10 <sup>15</sup>	1 X 10 <sup>20</sup>
<b>Sum Total:</b>		<b>6.6 X 10<sup>6</sup></b>	<b>1.11 X 10<sup>10</sup></b>	<b>1.01 X 10<sup>15</sup></b>	<b>1 X 10<sup>20</sup></b>

Thus, it follows that in the example population (Table 3) of over a million constructs (equally represented in the DNA), a single construct expressing 10<sup>5</sup> copies of RNA per DNA copy will increase to approximately 99% of the total expressed RNA sequences in

- 5 two passages. Using similar procedures in combination with drug and/or hormonal stimulation, and after consideration of the possible transcription factor binding sites within the sequence family (Figs. 5 & 6), it is within the intended scope of the invention to select for hormonal or pharmacological controls of transcription such as have been described herein. The factors contributing to the outcome are not only the input constructs, but recombinants  
10 and mutants as well. These secondary contributors to molecular diversity will be enhanced if multiple rounds of infections are allowed to occur, as oftentimes the difference between a particular transcription factor being able to bind (or not) may depend upon a single base change. Because viral infection is progressive and competitive, molecular evolution can be used to generate gene constructs *de novo* in the tissue culture dish in short time periods.  
15 Advantageously, the use primers to generate amplified fragments with uniquely complementary cohesive ends (i.e., that the ends will preferably only hybridize with the intended 5' and 3' fragments) to ligate three or more fragments as taught in this invention improves the potential for obtaining a diverse library.

Although the examples particularly point out a transcriptional promoter as the  
20 product of the process, the skilled artisan can appreciate that a particular selection technique can be applied to other *cis*- and *trans*-acting genetic sequences as well. Although a virus is used to propagate the selective advantage of a preferred embodiment, it can also be appreciated that any selective screen, such as drug selection, cell survival, phenotypic selection, cell sorting, antibody selection, and the like (see Ausuble et al., *supra*) could be

substituted without changing the intended scope of the invention. Likewise, transfection or cell fusion could be used in place of viral infection. Furthermore, substitution of different viruses, retrotransposons, or functional groups are likewise within the intended scope of the invention. The described embodiments are to be considered only as illustrative and not 5 restrictive, and the scope of the invention is indicated by the claims rather than by the narrative description. All references and publications, cited herein, are incorporated by reference into this disclosure.

Like the embodiments detailed above, the method of library production is also conducive to assembly and transfer of genetic material directly into eukaryotic cells, saving 10 the step of propagation in bacteria that is standard in bacteria. An advantage of direct transfer of the libraries of this invention to eukaryotic cells, including the exemplary retroviral vector producer cells, is that certain essential *cis*-acting structural features will be under positive selection (i.e., if they are not present, the molecule will be lost due to its non-functionality). As discussed above, it is often advantageous to eliminate bacterial and plasmid DNA 15 sequences, endotoxin, and other bacterial contaminants by introducing the constructs directly into eukaryotic cells.

In addition to providing a method for constructing complex DNA molecules efficiently (as in the examples of three piece and six piece constructs), the methods of this invention permit the assembly of constructs that are larger than those conventionally 20 propagated in *E. coli*. Examples of these types of vectors include adenovirus vectors, herpes simplex vectors and artificial minichromosomes. In order to insert genes into such vectors that are too large for conventional molecular cloning procedures, in the past it was often necessary to resort to *in vivo* recombination, wherein the genes of interest are cloned into a suitable vector and the flanking homologous regions are used to target the foreign genes to a 25 homologous site within the larger viral or minichromosome vector. However, the methods of this invention permit PCR fragments of any size (up to the limits of PCR capability, 20-30 kb per fragment) to be joined together. Thus, it is feasible to precisely construct adenovirus vectors by amplifying larger sequences, and combining them by ligation. For example, several sections of adenovirus (5-10 kb each) can be ligated using the methods of this 30 invention, up to for example, about 37 kb, and then transformed directly into human cells. Only the correctly recombined vectors are capable of replicating. Hence, the DNA is autoselecting. A similar procedure is used for generating herpes virus vectors, which are

approximately 150 kb. The precision of the methods of this invention permit non-essential-viral genes to be more easily eliminated from the construct. After transfection into appropriate cells, the DNA replicates and virus particles are formed.

Some special considerations apply to larger vectors, however. First, it is  
5 desirable to use enzymes that do not cut within the large DNA fragments. To prevent excessive fragmentation of the DNA by internal sites, it is desirable to use enzymes that cut rarely or infrequently, such as CpG-containing enzymes recognizing six bases, or enzymes such as *Sap1*, recognizing seven bases and digesting a three bp overhang (thus permitting up to 32 fragments to be joined in order). It is also desirable to avoid shearing the DNA once  
10 large segments have been joined by ligation. One method of avoiding shear is to add the transfection agent, such as Superfect™ reagent (dendrimers, Qiagen) or Lipofectamine™ (liposomes, Life Technologies, Gaithersburg, MD) directly to the ligation reaction, and then add the cells to be transfected to the mixture. This, or a similar method avoids the need to physically move the ligated DNA, and thus prevents shearing. Another method is to add a  
15 DNA condensing reagent (dendrimers, polycations [such as polyethyleneamine] histones or liposomes) directly to the DNA ligation reaction, and then move the DNA by pipette after it has condensed (thus reducing shearing of the DNA). Once inside the cell, viral DNA can replicate (as in the examples of partially replication-competent adenovirus and herpes simplex virus vectors).

20 Artificial minichromosomes have been under development for years. True artificial chromosomes require a centromere, at least one origin of DNA replication, and in the case of linear molecules, telomeric repeats at the chromosomal termini. In addition, to be very effective it is desirable to have a selectable marker gene, one or more therapeutic genes, and/or reporter genes.

25 In reality, the use of minichromosomes has been delayed by the inability to effectively manipulate the larger DNA molecules *in vitro*. Yeast and bacterial artificial chromosomes have been used with little success in mammals, and the addition of telomeres to the ends of linear chromosomes is also a special problem, as there is no prokaryotic host that can tolerate large linear DNA. The methods of this invention offers the opportunity to  
30 assemble human or mammalian minichromosomes *in vitro*, by using large segments (10-30 kb) of synthetic, gene-amplified DNA as ligation starting materials. For example, up to 32 *Sap1* fragments (up to 30 kb each, containing the essential *cis*- and *trans*-acting sequences),

or 512 shorter *Hgal* fragments can be combined using these methods. As with the other examples, several enzymes suitable for this invention (e.g., such as class IIS enzymes) can be combined (possibly with different termini lengths) to simplify the task. The methods of this invention also facilitate construction of telomeric repeats, because the constructs of this  
5 invention do not need to be circular. Thus, the methods of this invention can be used to make telomeres of any length, by adding additional segments onto the ends of molecules. One way to do this is using self assembling genes that employ a repeating overhang sequence (self-complementary molecule, such as AG-3' at one end, and CT-3' at the other end), permitting the telomeres to be lengthened to the extent desired by adding the required molar excess of  
10 the telomeric repeat-containing fragment. This technique gives the investigator some control over the relative length of the telomeres, although the self-complementarity indicates that many repeats will be lost due to self-ligation. This can be alleviated by using higher starting concentrations of DNA to favor inter-molecular ligations over intra-molecular ligations (e.g., >20 µg/ml starting concentration of DNA).

15 A two fold molar excess of telomeric fragments gives approximately twice the average length of telomere as a strictly 1:1 molar ratio of all fragments. By using a higher molar ratio of shorter telomeric repeats it is possible to give greater uniformity to the overall length of the molecules, which will vary from one terminus to the other. Thus, in addition to providing a way to build large molecules with precision, the methods of this invention  
20 provides for a way to control the telomere length (or potential life-span) of the artificial chromosome. To prevent damage during handling, the minichromosome DNA can be condensed with polycations, adenovirus particles, dendrimers, histones, or liposomes prior to transfection, similar to larger viral vectors.

The methods of this invention can be used to create recombinant virus. One  
25 example of this is an adenovirus vector self-assembling gene system. This system can include three parts: 1) vector; 2) helper virus; and 3) helper cells. The vector part is a self-assembling fragment set of at least three fragments comprising the essential cis-acting sequences (left and right inverted terminal repeats, which are the 103 bp at both ends of the genome that are required for replication [ITRs] and packaging sequences [Y, base pairs 194-  
30 358] and central 'baggage' area, comprising one or more self-assembling fragments including therapeutic genes, marker genes, and reporter genes. The baggage area is thus flanked by the cis-acting sequences in the vector. Because the synthetic oligonucleotide sequences

comprising the 5' and 3' termini of the helper virus are not phosphorylated, they will not ligate together creating multimers. Thus, the Ad5 vector region will assemble only into monomers. The helper virus part comprises all Ad5 trans-acting genes except for the E1A and E1B genes. The helper virus part has no cis-acting sequences, and it is amplified in several sections. In this preferred embodiment, the virus is amplified using primers that exclude the ITRs, packaging region and E1A&B genes. The helper virus is digested by *Sap1* digestion, creating seven uniquely terminated fragments comprising the trans-acting viral genome, with dephosphorylated, blunt 5' and 3' ends on the terminating fragments. The primers are designed so as to amplify the internal virus sequences without changing them, except for the 5' and 3' ends of the virus. The PCR-amplified fragments are digested with *Sap1* and are religated in their natural order after gel isolation and Qiagen column purification. The 5' end of the helper virus genome starts at 3.2 kb (in the E1A gene) so as not to overlap the vector sequences, which could otherwise cause replication competent adenovirus (RCA). Because the 5' and 3' ends of the helper virus do not contain *Sap1* sites, they remain intact after digestion with *Sap1*. Because the synthetic oligonucleotide sequences comprising the 5' and 3' termini of the helper virus are not phosphorylated, they will not ligate. Thus, the Ad5 helper virus genome assembles only into preferred monomers during ligation.

In a preferred embodiment, non-essential genes are deleted from the Ad5 genome by means of the method of self-assembling genes. In another preferred embodiment, the helper virus genome is approximately 30 kb after deletion of E1A, E1B and E3 gene sequences from the helper virus, and it is amplified as a single long fragment using the eLONGase Amplification System (Life Technologies or a similar strategy for creating long PCR fragments with high fidelity). It is not of great importance that occasional PCR errors may occur, because multiple copies of the Ad5 helper virus are transfected into target cells, thus providing trans-complementation. The helper cells are preferably 293 cells, a human kidney cell line expressing E1A and E1B genes (ATCC). The vector part and the helper virus part are combined in equimolar ratios after ligation has been performed separately on each fragment set. The Superfect protocol (Qiagen) is used to transfet the vector part and the helper part into the helper cells. The helper cells lyse, releasing high-titer adenovirus particles that are capable of infecting a variety of human cells. The resulting defective virus is incapable of forming RCA, and it transmits up to 34 kb of foreign genes in the baggage area. Unlike conventional Ad5 vectors that require separate constructs for *E. coli* propagation of

insert genes, and recombination in vivo, the present vectors are relatively easy to make and provide a precise, safe alternative to first generation and second generation adenovirus vectors.

Exemplary methods for producing self-assembling vectors and genes are provided below. Further, the Examples provide methods for producing libraries of nucleic acid sequences using the methods of this invention. A number of nucleic acid sequences identified using the methods of this invention are described. The examples provided below are exemplary and not limiting. All references and publications provided herein are incorporated by reference into this disclosure.

10

**Example 1**  
**Three-Piece Gene Self-Assembly with 100% efficiency**

Using 6 primers (SEQ ID NOS:24 and 63-67), three PCR fragments were amplified from templates VLMG (SEQ ID NO:22) and VLBN (SEQ ID NO: 1). PCR reactions were carried out using the hot start technique, according to the manufacturer's instructions (Perkin Elmer) using *Pfu* DNA polymerase (Stratagene). To amplify specific portions of the above templates, each primer contained a class IIS enzyme site capable of digesting a unique overhanging end that was complementary to only one other terminus in the subsequent ligation. The class IIS enzymes used were *Bpm*1 and *Eco* 57I (the latter was used to copy a fragment that contained an internal *Bpm*1 site). The reactions were carried out as follows: 1) the lower reaction was assembled according to the protocol for PCR Gems (Perkin Elmer); 2) the lower reaction was heated to 80°C, 5 min, then cooled to 4°C for 5 min; 3) the upper reaction was prepared according to PCR Gems protocol and was added to the lower reaction (separated by cooled wax). The primer concentration was 0.3 μM (final). The dNTP concentration was 200μM (final). 5 Units of *Pfu* polymerase was used. All fragments were amplified using the following conditions: 96°C, 45 sec; (then followed by 30 cycles of the following) 96°C 45 sec, 52°C 45 sec, 72°C, 6 min; then followed by a single incubation at 72°C for 10 min; then hold at 4°C. All fragments were successfully amplified. The PCR fragments were purified using the Qiaquick PCR purification protocol (Qiagen). The fragments were digested with an excess of the appropriate restriction enzyme (*Bpm*1 or *Eco*57I). The digested fragments were run on a 1% agarose gel and were excised using minimal irradiation from a hand-held 365 nm ultraviolet light. The fragments were purified

using the Qiagen Qiaquick Gel Purification Protocol. The fragments were ligated at an equimolar ratio at a concentration of >20 $\mu$ g/ml with T4 DNA ligase (Boehringer Mannheim) overnight at 4°C. Competent *E. coli* SCS110 cells (Stratagene) were transformed with the ligated DNA. Eight colonies were characterized by restriction enzyme analysis, and all eight 5 contained the correct order and orientation of the three fragments. The experiment was repeated independently by another investigator, and the same result was obtained (8/8=100%). Thus, the procedure resulted in a high percentage of correctly assembled vectors.

This three-piece vector was VL $\Delta$ BP. The deletion extended from the distal 10 enhancer region to the TATA box near the start of transcription. The deletion region was a pair of *Bpm*1 sites that permitted U3 sequences to be cloned into the insert.

One validated *E. coli* clone of VL $\Delta$ BP was transfected into retroviral helper 15 cells. After 48 h, the vector was transduced into amphotropic helper cells. After selection for two weeks with the drug G418, drug resistant colonies were grown up in a mass culture and the vector was transduced from the amphotropic helper cells into a human HT1080 cell line (ATCC, Rockville, MD). Surprisingly, even with a large deletion in the LTR promoter, the basal TATA box-containing VL $\Delta$ BP was transmitted as a retrovector and was permanently inserted into the human cell line, thus establishing the validity of the self-assembly technique for the construction of functional eukaryotic vectors.

20

#### **Example 2** **Production of a Six Piece Self-Assembling Expression Vector**

Due to the high efficiency of the gene self assembly process for the three piece 25 assembly, a complex vector containing six fragments was constructed. The results here were extended to determine whether such a self-assembled vector would also have biological activity in human cells without being cloned and grown in a prokaryotic cell.

Six fragments were individually constructed by PCR using three different templates and twelve primers (as illustrated in Fig.8). The primers used three different class 30 IIS enzymes. The enzymes were chosen so as to give 2 base pair, 3'-overhanging ends. Three enzymes were used in order to avoid the use of enzymes that had additional sites internal to the fragments being amplified. Thus, *Bpm*1 was used unless there was an internal *Bpm*1 site. If such a site existed, *Eco*57I was used. If there was also an internal *Eco*57I site, then *Bsr*D1

was used. However, it is alternatively possible to use an enzyme such as *Eam*11041, where the *Eam*11041 sites in the primers are unmethylated (therefore susceptible to digestion by the enzyme), and wherein the <sup>m5</sup>dCTP analog of dCTP is used in the PCR reaction, methylating all internal sites (and protecting them from digestion by *Eam*11041), as suggested by Padgett  
5 and Sorge, 1996, *supra*, and incorporated herein by reference.

Using 12 primers, 6 fragments were amplified from 3 templates: pBK-CMV (SEQ ID NO:26) , pVLMB (SEQ ID NO:23) and pVLOVhGH-900 (SEQ ID NO:21).

Fragment 1 was amplified from pBK-CMV using primers 1 and 2 (SEQ ID NOS:31 and 32).

Fragment 2 was amplified from pVLMB using primers 3 and 4 (SEQ ID NOS:33 and 34).

10 Fragment 3 was amplified from pVLOVhGH-900 using primers 5 and 6 (SEQ ID NOS:35 and 36). Fragment 4 was amplified from pVLMB using primers 7 and 8 (SEQ ID NOS:37 and 38). Fragment 5 was amplified from pVLMB using primers 9 and 10 (SEQ ID NOS:39 and 40). Fragment 6 was amplified from pVLMB using primers 11 and 12 (SEQ ID NOS:41 and 42). PCR reactions were carried out using the hot start technique, according to the  
15 manufacturer's instructions (Perkin Elmer Ampliwx PCR GEMS 100). The lower reaction was heated to 80 ° C for 5 min, then cooled to 20 °C for 5 min. The upper reaction was prepared according to PCR gems protocol and was added to the lower reaction (separated by cooled wax). The primer concentration was 0.3 micromolar (final). The dNTP concentration was 200 µM (final). 5 U of *Pfu* polymerase (Stratagene) was used per reaction. 100 ng of  
20 template was used for each reaction 14 rounds of PCR amplification were used to reduce mutagenesis of the templates. The PCR cycling protocol was 96 °C 45 sec; then two cycles of (96°C 45 sec, 52°C 45 sec, 72°C 6 min); then 12 cycles of (96°C 45 sec, 58°C 45 sec, 72°C 6 min) followed by a 72° C soak for 10 min, then to 4°C hold.

The six PCR fragments were designed to self-assemble into a retro-vector after  
25 digestion with the correct class IIS restriction enzyme (Fig. 8). After transfection into retroviral helper cells, the vector DNA is transcribed as RNA by means of the cytomegalovirus immediate early promoter (fragment 1). This promoter replaces the retroviral or VL30 LTR in this vector. The RNA transcript region begins with the R and U5 regions of the Moloney murine leukemia virus (MoMLV) LTR, the viral packaging signals  
30 ( $\Psi$ ) region of MoMLV, the packaging enhancer ( $\Psi^+$  ) region of mouse VL30 and the IRES region of EMCV fragment 2. Fragment 3 consists of the human growth hormone (hGH) cDNA sequence. Fragment 4 consists of the SV40 virus early region promoter driving

expression of the neomycin phosphotransferase (neo) gene. Fragment five consists of the (+)-strand primer binding site of the MoMLV LTR, the U3 region of the MoMLV LTR, the repeat (or R) region, and a portion of the U5 region. Fragment 6 consists of the PBR322 plasmid origin of replication.

5

Fragment 1: CMV early region promoter

Template: pBK-CMV plasmid DNA (Stratagene, LaJolla, CA) *Bpm1* (SEQ ID NO:26)

PCR primer 1 (SEQ ID NO:31)

10 GACTAACCTTGATTCCACTGGAGCCGTATTACCGCCATGCATTAGTTATTAATAG  
PCR primer 2 (SEQ ID NO:32)

GACTAACCTTGATTCCACTGGAGTAATTGCGGCTAGCGGATCTGACG

Fragment 2: R-U5-Psi-Psi(+) -IRES *Bpm1*

15 Template: pVLMB plasmid DNA (SEQ ID NO:23)

PCR primer 3: SEQ ID NO:33

GACTAACCTTGATTCCACTGGAGACACTTGACCTCTACCGCGCCAGTCCTCCGAT  
TGACTGAGTCG

PCR primer 4: SEQ ID NO:34

20 GACTAACCTTGATTCCACTGGAGGGATCCGCGCCCATGATTATTATCG

Fragment 3: human growth hormone (hGH) *Bsr* DI

Template: pVLCNOVhGH plasmid DNA (SEQ ID NO:21)

PCR primer 5: SEQ ID NO:35

GACTAACCTTGATTCCAGCAATGTCGGTTAGCTTGTTCCTTACTGTTGTC

25 PCR primer 6: SEQ ID NO:36

GACTAACCTTGATTCCAGCAATGTTAGGACAAGGGCTGGTGGGCACTGG

Fragment 4: SV40 early promoter-neomycin phosphotransferase

Template: VLMB plasmid (SEQ ID NO:23)

30 PCR primer 7: SEQ ID NO:37

GACTAACCTTGATTCCACTGGAGGGTCGACCCTGTGGAATGTGTGTCAG

PCR primer 8: SEQ ID NO:38

GAACCTTGATTCCACTGGAGAATCTCGTATGGCAGGTTGGCGT

Fragment 5: MLV(+)PBS-U3-R-U5

5 Template: VLMB plasmid (SEQ ID NO:23)

PCR primer 9: SEQ ID NO:39

GAACCTTGATTCCACTGAAGAGATTTATTAGTCTCCAGAAAAAGGGGGG

PCR primer 10: SEQ ID NO:40

GAACCTTGATTCCACTGAAGCCCCAAATGAAAGACCCCCGCTGACG

10

Fragment 6: PBR322 origin of replication

Template: VLMB plasmid (SEQ ID NO:23)

PCR primer 11: SEQ ID NO:41

GAACCTTGATTCCACTGGAGCCGGACGGAATCGTAATCTGCTGC

15

PCR primer 12: SEQ ID NO:42

GAACCTTGATTCCACTGGAGTTCTGAGGCGCGCATCTGGCG

*Procedure:* The twelve primers were prepared by the following procedure: 1) oligonucleotides were synthesized with trityls off. After deprotection and lyophilization, the samples were resuspended in 5 microliters deionized formamide and loaded onto a polyacrylamide gel (12% polyacrylamide, 250V). The samples were excised under short wave UV irradiation and eluted overnight in 600 microliters of sample elution buffer (0.5 M ammonium acetate, 10 mM Mg acetate, 1 mM EDTA, 0.1% SDS). The contents were loaded onto a BioRad Chromatography column (Cat. # 732-6008) and centrifuged into an Eppendorf tube at low speed (2000 RPM, 5 min). After washing the column with 500 microliters TE buffer (10 mM Tris, 1 mM EDTA), pH 8.0 and recentrifugation (2000 RPM, 5 min), the pooled eluate was ethanol precipitated, washed with 100% ethanol, resuspended in TE buffer and quantitated by spectrophotometry of a small sample, which was then discarded.

Fragments were cleaned using the Qiaquick PCR cleanup procedure. The fragments were digested with their respective class IIIS restriction enzyme. The digested fragments were run on 1% agarose gels, and the fragments were excised and cleaned using the Qiaquick gel cleanup procedure. Fragments were combined in an equimolar mixture and

ligated overnight at 4° C with T4 ligase and ATP. An analytical gel was run with the ligated DNA, as well as with controls including unligated fragments and ligated fragments with a single fragment missing. As opposed to the controls, the complete ligation included bands equivalent to the full-length supercoiled monomer (referred to as GENSA 981, SEQ ID NO:29), as well as bands possibly representing multimers (up to six bands were observed).

In order to assess the efficiency of the method, eleven nanograms of DNA were transfected into SCS1 supercompetent cells. Thirteen kanamycin resistant colonies were harvested, and plasmid DNA preps indicated 10 out of thirteen that appeared to be the correct length. All ten gave the expected bands when digested with *Pst*1, *Sna*B1, and *Bam* HI. 1.35 µg of the ligated DNA was purified by phenol-chloroform-isoamyl alcohol extraction, followed by two extractions with chloroform-isoamyl alcohol, and was precipitated in ethanol. The DNA was washed in 70% ethanol and re-suspended in 50 µl of sterile phosphate buffered saline (for transfection). The DNA was transfected (using the Qiagen Superfect protocol) into HTAm1 (amphotropic human helper cells). 24 h after transfection, the target cells were washed and fresh culture media was added. 48 h after transfection, the supernatant from the vector producer cells was filtered (0.45 µm, Nalgene) and transferred to PG13 helper cells (ATCC) and HT1080 human fibrosarcoma cells. This procedure was repeated after 72 h. 48 h after transduction, recipient cells were started on G418 drug selection (500 µg/ml). The appearance of G418 drug-resistant colonies on transduced PG13 and HT 1080 cells after 6 days of selection indicated successful transmission via retrovirus particles. The transfect HTAm cells were also selected with G418. After six days of drug treatment, 45 colonies of resistant cells were counted. Thus, the six fragment gene assembly was effectively transmitted and expressed as either a DNA (transfection) vector or a retro-vector.

25

**Example 3**  
**Design and Construction of Single LTR Vectors**

*Background:* In order to manipulate the interior of the VL30 LTR sequences using a promoter rescue technique, single LTR vectors were constructed. The mouse VL30 element NVL-3 was used as the starting material as it is constitutively and abundantly expressed in most mouse tissues. Single LTR vectors are circular and behave as if they contained two LTRs. Thus, in these vectors RNA transcription begins at the start of the R region (see Fig.

3B), and continues through the polyadenylation site after completing the second round of transcription of the R sequences (Fig. 3A). In previous studies, these vectors were expressed transiently in vector producer cells and the DNA did not integrate into cell DNA as a standard two LTR vector. Therefore, the vectors were usually passed to a second complementation 5 helper cell line via retroviral transduction of the vector RNA transcribed in the first helper cell. This process resulted in the vector regenerating a correct (two LTR) structure upon integration into the recipient cell DNA.

*Experimental method:* The plasmid pNVL-3 (SEQ ID NO:25, kindly provided by Dr. J. Norton Manchester, UK), containing a complete copy of the NVL-3 (mouse VL30) genome (Adams *et al.*, 1989), was digested with *Xho*1 (which cuts in the LTRs), releasing the 4.27 kb VL30 genome with one copy of the LTR. This fragment was circularized using T4 DNA ligase and ATP. The circular DNA was linearized by digestion with *Sna*BI, 187 bp from the 3'-LTR. A 2.3 kb fragment containing the SV40 virus early region promoter and the 15 aminoglycoside phosphotransferase (*neo*) gene, together with the PBR322 plasmid origin of replication, was excised from the BAG retrovirus vector (Price *et al.*, *Proc. Natl. Acad. Sci.* 84:156-160, 1987, kindly provided by C. Cepko, Cambridge, MA). BAG is also obtainable in a retrovirus helper cell line from American Type Culture Collection (ATCC), Rockville, MD by digestion with *Xho*1 and *Bam*HI. This fragment was blunted with T4 DNA 20 polymerase and dephosphorylated with calf intestinal alkaline phosphatase (CIP). The fragment was then ligated to the linearized *Sna*BI fragment of NVL-3. The resulting plasmid (containing a circularly permuted NVL-3 genome with the SV-*neo-ori* region) was designated VLSNO2 (SEQ ID NO:30).

In order to facilitate the switching of LTR sequences by means of the class IIS 25 enzyme *Bpm*1, VLSNO2 was digested with *Bpm*1 (six sites). The region containing four *Bpm*1 sites was removed and replaced with a 19 bp linker (SEQ ID NOS: 1 and 52, see below), 921 bp beyond the LTR. The linker contained *Sna* BI, *Cla*1 and *Bam* HI cloning sites.

Linker (top strand): 5'-TACGTATCGATGGATCCGA-3' (SEQ ID NO:51)  
30 Linker (bottom strand): 5'-GGATCCATCGATACTGTAAG-3' (SEQ ID NO:52)

The remaining two of the *Bpm1* sites had complementary ends, which permitted their ligation and resulted in eradication of all *Bpm1* sites within the resulting vector VLSNO3 (SEQ ID NO:20).

In order to facilitate reporter/therapeutic gene function, a 3.7 kb fragment containing the internal ribosome entry site (IRES) from encephalocytocarditis virus, together with the  $\beta$ -galactosidase reporter gene, was excised from the plasmid pVLSAIBAG (kindly provided by Mr. James Grunkemeyer, Omaha, NE) by means of a partial digestion of the plasmid with *Bam* HI. This region was inserted into the *Bam* HI site of VLSNO3, resulting in the vector VLSNOSIB (SEQ ID NO:14).

A second reporter construct, pVLSNOG (5774 bp, SEQ ID NO:19) contained the green fluorescent protein (GFP, Clontech, Palo Alto, CA) gene was constructed by inserting a *Bgl*2-*Bcl*1 fragment (800 bp) from plasmid pGFP-N1. This sequence, containing the GFP gene, was treated with mung bean exonuclease and inserted into the unique *Sna* B1 site of pVLSNO3.

In order to enhance GFP fluorescence from the reporter plasmid pVLSNOG, the serine-65 codon in the GFP gene was mutated into threonine by a site-directed mutagenesis procedure with the Transformer™ Site-Directed Mutagenesis kit from Clontech. A *Bpm1* site in the GFP gene (threonine-9) was mutated at the same time without changing the amino acid (ACT to ACA). The resulting plasmid was pVLSNOGM (SEQ ID NO:18).

An *Nco*1-*Xho*1 fragment (585 bp) from plasmid pG1IL2EN (kindly provided by Dr. Steven Rosenberg, Bethesda, MD), containing the internal ribosome entry site (IRES) from encephalomyocarditis virus (EMCV) was inserted into the *Apal* site upstream of the GFP gene in pVLSNOGM, resulting in pVLSNOGMI (SEQ ID NO:17). Both insert and plasmid fragments were blunted with mung bean exonuclease. One variant version of pVLSNOGMI with an IRES tandem dimer was also constructed and designated pVLSNOGMI2 (SEQ ID NO:16).

Oligonucleotides (SEQ ID NO:53 and 54) containing a splice acceptor (SA) of AKV virus (in bold) was inserted into pVLSNOGMI at the unique *Sac* 2 site just before the IRES, resulting in pVLSNOGMIS (SEQ ID NO:15).

Oligo: (SEQ ID NO:53)

5' - GGCCGCT**A**CTAATAGCCCATTCTCC**A**GGTACGTAGC - 3'

3' - CGCCGGCGATTGATTATCGGGTAAGAGGTTCCATGCAT - 5'

(SEQ ID NO:54, bottom Oligo)

**Recovery of LTR promoter sequences from mouse CD4+ T-helper cells**

In order to facilitate the recovery of VL30 promoter sequences expressed in mouse T-helper cells, a mouse CD4+ T-helper cell cDNA library (Stratagene, San Diego, CA, Catalog # 937311) was screened by plaque hybridization. Approximately  $2 \times 10^4$  bacteriophage  $\lambda$ -ZAP clones were plated on a lawn of *E. coli* cells according to the manufacturer's instructions. Two nylon filters were sequentially layered onto the lawn of *E. coli* cells and bacteriophage. The filters were hybridized to a  $^{32}\text{P}$ -labelled (Prime-It RmT Random Primer Labeling Kit, Stratagene), 4.2 kb internal *Xho*1 fragment of NVL-3 (containing the NVL-3 genome). 55 plaques (or approximately 0.3% of the total phage) reacted positively on both filters. 18 VL30 cDNA sequences were cloned from the plate, which was used to identify U3 promoters that are actively expressed in the RNA of mouse T-cells. Five of the 18 clones contained intact U3 sequences, representing four of one molecular species, named TH1 (SEQ ID NO: 2) and one of another species, named TH2 (SEQ ID NO: 3) also provided in Fig. 5. TH1 contained approximately 120 bp more DNA than did TH2. Because TH1 was more abundant (4 out of 5 clones), the additional sequences in the enhancer region were implicated to be a possible reason for the stronger expression in mouse T cells. Examination of the known and putative transcription factor binding sites in the VL30 LTR (Hodgson, 1996, chapter 4, Fig. 4.2 *supra*) revealed several interesting features of TH1 and TH2. First, the extra sequences of TH1 that were missing in TH2 included an extra copy of the enhancer repeat region as well as a potential retinoid (RAR/RXR) binding site. Several transcription factor binding sites in the enhancer repeat region that differed between the two elements included: a cyclic 3'-5'AMP response element (VLCRE, a potential CREB/jun binding site), a serum response element (SRE), and a potential NF1/IL6 binding site (although there were additional sites for these factors in other enhancer repeats). These factors could possibly explain why VLTH1 appeared to be expressed at higher levels, both in the source cells and into transduced cells. Together, the VL30 sequences represented 0.3% of the mRNA expressed in the T cells, and TH1 appeared to be most abundant VL30.

**Sequencing Primers:**

(SK, SEQ ID NO:49) 5'-CGCTCTAGAACTAGTGGATC (20 mers, Tm 60°C).

(T7, SEQ ID NO:50) 5'-GTAATACGACTCACTATAGGG (21 mers, Tm 60°C).

5      **Seamless Rescue of T cell promoters using class IIS restriction enzymes**

Two sets of primers containing offset *Bpm*1 restriction sites were designed and synthesized. One set was for amplification of the plasmid sequences, and another was for the amplification of the inserts.

10     **Insert Primers: (*Bpm*1 site bold)**

ITA (43 mer, Tm: 67.2 °C, SEQ ID NO:45)

CGATCCACT**GGAG**CTGGAGGCCACCCCCTCCATCTAGAGGT

## 15     ITB (43 mers, Tm: 66.3 °C, SEQ ID NO:46)

CGTCCT**GGAG**GACCTCTAGATGGAGGGGTGGCTCCGAG**Vector primers: (*Bpm*1 site bold)**

VLA (43 mers, Tm: 68.2 °C, SEQ ID NO:47)

CGCAACC**CTGGAG**ACCTCTAGATGGAGGGGTGGCTCCGAG

## 20     VLB (43 mers, Tm: 66.3 °C, SEQ ID NO:48)

GCAGGAC**CTGGAG**CTGACCGTCGAGACTCCTCTACCTGTGCT

To amplify vector sequences more efficiently, vector templates were shortened by deleting marker genes from vectors. pVLSNOSIB (SEQ ID NO:14) was cut with *Kpn* 1 and a 4201 bp fragment containing β-gal gene was removed. The remaining vector has 3923 bp.

The U3-promoter inserts (357 bp for TH1 and 240 bp for TH2) were PCR-amplified from TH1 and TH2 promoters with primers ITA and ITB. The vector cassettes (~4.2 kb for pVLSNOSIB and ~3.7 kb for pVLSNOGMIS) were PCR-amplified from the shortened vector templates using primers VLA and VLB, (*supra*). The PCR-amplification was done with high-fidelity *Pfu* DNA polymerase from Stratagene (La Jolla, CA). The amplified products were gel-purified (1% agarose gel). The inserts were then cut with *Bpm* 1 to produce complementary ends. The vector cassette products were phosphorylated with

PNK, then circularized with T4 ligase, and transformed into SCS 110 cells. Recovered plasmids were then digested with *Bpm* 1 and treated with CIP to produce complementary ends. *Bpm* 1 treated inserts and vector cassettes were ligated, and T-cell tissue-specific VL 30 vectors VLTH1 and VLTH2 were produced. The marker  $\beta$ -gal gene and GFP gene were put back into those vectors at the original unique sites *Kpn* 1 and *Sal* 1 respectively.

#### Transmission and expression of single LTR vectors and T cell U3 sequences

Vector DNA constructs were transfected into GP+E86 retroviral helper cells (Markowitz et al, 1988, *supra*) using the Lipofectamine protocol (Life Technologies, Gaithersburg, MD). The culture media from these cells (supernatant), containing defective transducing particles (72 h post-transfection), was transmitted to PA317 (Miller, US Patent, cited *supra*) amphotropic helper cells, using Lipofectamine to enhance transduction efficiency (Hodgson et al., 1996. Synthetic Retrotransposon Vectors and Gene Targeting pp. 3-14, in : Felgner et al., eds. *Artificial Self-Assembling Systems for Gene Delivery*. American Chemical Soc. Books, Washington, D.C.). A similar procedure was used to transmit VLTH1 and VLTH2 to the PG13 helper cell line (Miller et al., 1991. *J. Virol.* 65:2220-2224). 24 h post-transfection, the recipient cells were selected with the drug G418 (500 $\mu$ g/ml, 2 weeks) to enrich for stably transduced cell populations.

All of the single LTR vectors, including VLTH1 and VLTH2 were transmitted by this method, indicating that single LTR vectors can be used for promoter switching and yet revert to dual LTR vectors after a single passage. Vectors VLSNO2, VLSNO3, and VLSNOSIB were then titered on NIH 3T3 cells (using the PA317 vector producer cell lines). VLTH1 and VLTH2 vectors were titered on human HT1080 cells (PG13 cell lines). Surprisingly, all of the single LTR vectors were transmitted effectively. However the titers of stably transduced TH1 and TH2 cell lines were  $5.5 \times 10^2$ - $1.1 \times 10^3$  TU/ml, compared to  $0.4$ - $3.0 \times 10^4$  TU/ml for the VLSNO2, VLSNO3 and VLSNOSIB cell lines. Thus, switching from the NVL-3 transcriptional promoter (originally isolated from NIH 3T3 fibroblast cells) to VL30 promoters derived from T helper cells, appeared to have a negative effect on RNA expression in fibroblast cells, as determined by the transmissibility of the RNA.

In order to study the usefulness of rescued promoters as DNA transfection vectors (as opposed to retro-vectors), VLSNOSIB, VLTH1 and VLTH2 were also transfected into a number of cell lines (using Lipofectamine), including NIH 3T3, PA317, GP+E86,

PG13, HT1080, SW480 and HeLa (available from ATCC). RNA expression in these cell lines is shown in Table 4, wherein gene expression from the LTR promoter (as determined by  $\beta$ -gal staining) is normalized to VLSNOSIB (100).

Cell line:	NIH 3T3	PA317	GP+E86	PG13	HT1080	SW480	HeLa
<b>Vector:</b>							
VLSNOSIB	100	100	100	100	100	100	100
VLTH1	39.3	18.7	0.1	21	25.5	156	156
VLTH2	28.6	7.1	5.5	11.5	46.8	82	156

5 Table 4. Transient expression of a  $\beta$ -gal marker gene by three VL30 promoters: NVL-3 (VLSNOSIB), VLTH1 and VLTH2. Cells were transfected using the Lipofectamine procedure. Total blue cells were counted from each well in 6-well plates, and the number of blue cells from VLSNOSIB was normalized to 100%.

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The expression of both the VLTH1 and VLTH2 promoters was significantly reduced compared to VLSNOSIB in cell lines of fibroblastic origin, whereas in SW480 colorectal cancer cells and HeLa cells, it was comparable to or better than VLSNOSIB (the NVL-3 promoter). However, VLSNOSIB was expressed poorly in the non-fibroblastic cell lines, so a direct comparison was difficult to interpret. Unfortunately, the human T cell lines (Jurkat and MOLT4 [obtained from ATCC]) were not transfected by Lipofectamine, and they were poorly transduced by VLTH1 and VLTH2 retro-vectors. In the Jurkat and MOLT4 cells transduced with VLTH1 and VLTH2, only a small percentage (1-10%) of cells that were 15 stably transduced by the vectors stained positively for  $\beta$ -gal expression. However, the marker gene (neo) continued to be expressed from an internal promoter, as evidenced by drug 20 selection.

25 Taken together, the results demonstrated: 1) the ability of the promoter rescue technique to seamlessly capture functional transcriptional promoters from specialized cells; 2) the ability of single LTR vectors to introduce the rescued promoters into standard transducing vectors; 3) the ability of the rescued promoters to be expressed at differing levels in several different cell types, including T cells; and 4) screening and selection established the efficacy, or lack thereof, of individual promoter sequences.

30 Although the general method of promoter rescue was demonstrated by the foregoing experiments, the titers obtained from the sLTR VL30 vectors may not be useful where selection systems are not available.

Additional experimentation led to the development of a chimeric packaging signal, combining the essential packaging signal from Moloney murine leukemia virus ( $\Psi$ ), and the enhanced packaging signal ( $\Psi^+$ ) from a mouse VL30 element. A vector embodiment of this packaging system is VLMB (SEQ ID NO:23). One advantage of the chimeric packaging system was the elimination of retroviral *gag* gene sequences that were present in previous high-titer MLV-based vectors (viral *gag* sequences contribute to the generation of replication competent retrovirus outbreaks). The titers of VLMB-based vectors ranged from approximately  $1 \times 10^5$  to  $4 \times 10^6$  TU/ml.

10           **Construction of a cloning vector for promoter rescue**

Using pVLSNOGMIS as a template, and primers (SEQ ID NOS:28 and 68), a 6.4 kb plasmid fragment was PCR amplified (Using Hot Start Ampliwax PCR Gems 100, Perkin Elmer). 30 cycles of PCR were performed by following the manufacturer's instructions, with the following input conditions: lower reaction, 80° C, 5 min., then add 15 upper reaction and template, 96° C, 1 min. Each reaction vial contained 50 ng template, 0.5  $\mu$ M each primer, 200  $\mu$ M dNTPs and 5U (2 $\mu$ l) *Pfu* polymerase (Stratagene, LaJolla, CA). 30 repeating cycles of: 96° C, 45° sec; 50° C, 45 sec; 75 C, 1 min. A final incubation of 75° C, 10 min, then hold at 4° C. After amplification, the reactions were purified using Qiaquick PCR Purification Kits (Qiagen). The PCR products were digested with *Pac*1, heat inactivated 20 (65° C, 20 min) and ligated together using T4 DNA ligase (overnight at 4° C in a 5  $\mu$ l vol). The ligated DNA was transfected into SCS110 *E. coli* cells (Stratagene) with kanamycin (50  $\mu$ g/ml) antibiotic added to the agar plates. The cells were *dcm*<sup>-</sup>, *dam*<sup>-</sup> (to prevent methylation of *Bpm*1 sites). The resulting plasmid, pVLBPGN (SEQ ID NO:1, Figs 2 &3) has a deletion 25 in the U3 region of the LTR. A linker containing a central *Pac*1 site flanked by two outwardly-digesting *Bpm*1 sites occupies the site of the deleted U3 sequences. The *Bpm*1 sites enable the plasmid to be digested with *Bpm*1, resulting in two 2 bp 3'-overhanging ends that are complementary to the U3-derived RT-PCR inserts described below. The digested plasmid was purified free from the intervening linker sequences from an agarose gel after digestion with *Bpm*1, using the Qiaquick gel purification kit (Qiagen).

**Procedure for amplification of liver U3 promoter region**

Purified mouse liver total tissue RNA was purchased from Ambion, Inc., (Austin, TX). Total liver RNA was treated with RQ1 Rnase-free (Promega, Madison, WI). Using Perkin Elmer Gene Amp thermostable rTth reverse transcriptase RNA PCR kit (P/N 5 N808-0069), the following conditions for RT-PCR were used: RT-PCR A 70° (hot start); RT-PCR B, 95°C, 60 sec, then 35 cycles (95°m 10 sec, 58°C, 15 sec) then a final 58°C incubation for 7 min, then 4°C and hold. Additional conditions were: primer concentration 0.15 micromolar, template 100 ng/reaction, dNTPs 200 micromolar (final) and MgCL<sub>2</sub> 3.5 mM(final). The primers for insert amplification were SEQ ID NOS:28 and 68)

10 The amplified U3 sequences were purified using Qiaquick. The pVLBPGN plasmid was digested with *Bpm*1, isolated from a 1% agarose gel and purified using the Qiaquick method. The purified U3 sequences were ligated at 1:2, 1:4 and 1:6 molar ratios of VLBPGN plasmid:insert using T4 DNA ligase and a 5 microliter reaction volume overnight at 4°C (100 ng plasmid: 16 ng insert = 1:1 molar ratio). 1 microliter of each ligation reaction 15 was transformed into *E. coli* SCS 110 competent cells (Stratagene). 26 colonies were recovered in total. Out of 23 clones grown overnight in the presence of kanamycin, 20 had sequences that appeared to be mouse VL30 sequences, representing 10 different VL30 species (Fig. 6, SEQ ID NOS: 4-13). One of these (Hep 10, SEQ ID NO: 13) was transiently transfected into Hep G2 liver hepatocellular carcinoma cells. 48 h after transfection, intense 20 GFP fluorescence was observed, indicating strong expression of the Hep 10 U3 promoter region.

**Example 4**  
**Creating a combinatorial library of mouse VL30 U3 sub-regions.**

25 Using Fig. 7 and Hodgson, 1996, supra, Fig. 4.2 as a guide, the following three sub-regions of the VL30 U3 region were empirically established: Distal (1); medial (2); and proximal (3). Peaks of similarity were used to guide the following choice of primers: (+) primer binding site-5'-LTR boundary; ~80 bp (defines sub-region 1); ~80-210 bp (sub-region 30 2); ~210-430 (sub-region 3). The following primers were selected to amplify the vector VLBPGN or a similar VL30, NVL-3 LTR-containing vector:  
P1 (going left from the 5'-end of the LTR to amplify the plasmid)

(SEQ ID NO:55)

GACTAACCTTGATTCCACTGGAGTTT(CT)(CT)ATTCTTCATTCCCCACTTC  
TTCTT

P2 (going right from the 3'-end of the promoter region to amplify the plasmid)

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(SEQ ID NO:56)

GACTAACCTTGATTCCACTGGAGAATCTGGACCAATTCTATATAAGCCTG  
TGAAAAAATT

The six primers selected to amplify the inserts are as follows:

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Fragment 1, primer 1 (going right from the LTR terminus into U3) (SEQ ID NO:57)

GACTAACCTTGATTCCACTGGAGAAGAAGAAGTGGGAATGAAGAA

Fragment 1, primer 2 (going left from the end of fragment 1) (SEQ ID NO:58)

GACTAACCTTGATTCCACTGGAGATCTCTAGATGGGAGGG(GT)(CT)GGG  
CTC

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Fragment 2, primer 1 (going right from the left end of fragment 2) (SEQ ID NO:59)

GACTAACCTTGATTCCACTGGAGCTCGGAGCCCACCCCTCCCATCT

Fragment 2, primer 2 (going left from the right end of fragment 2) (SEQ ID NO:60)

GACTAACCTTGATTCCACTGGAGGGAGGCCCTATCTCAAAAATGTT

Fragment 3, primer 1 (going right from the left end of fragment 3) (SEQ ID NO:61)

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GACTAACCTTGATTCCACTGGAGTCTAACAAACATTTGAGATAAGGGCC  
T

Fragment 3, primer 2 (going left from the right end of fragment 3) (SEQ ID NO:62)

GACTAACCTTGATTCCACTGGAGTCACAGGCTTATATAG(TG)AAA

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100 ng of genomic DNA from *Mus musculus* is used as a template (the mouse genome bears 100-200 copies of VL30 elements). Standard PCR procedures for *Pfu* polymerase are used. Fragments are amplified 35 rounds of PCR to obtain single-copy genomic DNA amplification. Samples of Qiagen column purified DNA are examined on analytical agarose gels to determine the approximate size. The remainder of each reaction is digested with the appropriate enzyme and run on an acrylamide or agarose gel. The digested fragments are purified by standard gel purification procedures and are ligated to the plasmid fragment at an equimolar ratio of the four PCR fragments (three inserts and one plasmid). The ligation mix

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is transformed into *E. coli* SCS1 and is grown on kanamycin. The number of colonies is used to establish the size of the combinatorial library, and the pooled colonies are grown in *E. coli* and the DNA is harvested *en masse*. A dozen or more colonies are characterized by DNA sequencing to determine the approximate fidelity of the reaction. A library of 1,000 or more, 5 but preferably 100,000 or more members is used for combinatorial screening procedures.

**Screening the combinatorial libraries for expression in specific cell types using a replication defective helper virus**

The U3 library DNA is transfected into the desired target cells in which 10 expression is desired. Along with the library, approximately 25% of the total DNA should include retroviral helper sequences. The latter sequences can be a helper plasmid (such as pPAM3, Miller *et al.*, US Patent 4,861,719). The virus is amphotropic, permitting it to infect most human cells. The RNA from individual clones that are transcribed in the target cells will be packaged into retroviral virions made by the helper virus, and the virions can be harvested 15 as the cell free filtrate (0.45 mm) from the vector producer cells. This virus (containing the expressed sequences) can be transmitted to fresh target cells that do not contain helper virus. 48 hours after passage, the DNA form of the transcriptionally active clones will be integrated in the recipient cells, and these transcriptionally active loci will produce more RNA, and protein. After G418 drug selection to increase the proportion of cells expressing the vector 20 sequences, helper virus DNA is again transfected into the recipient cells, transforming them into vector producer cells. The virus from these cells should contain increased amounts of the RNA from clones that are transcriptionally active in those cells. Passage of the virus is continued for two or three rounds to permit recombination and mutation to take place, enhancing the effect of *in vitro* evolution of promoters. The actual degree of enhancement 25 attainable at each step is illustrated in Table 2 (*supra*). After several passages, the actual level of RNA expressed by several clones is determined by RNA blotting, or by the amount of a reporter gene expressed as protein (determined visually or by the appropriate assay). Because human cells do not naturally contain VL30 DNA or RNA, the sequences that remain in the human cells are those with the most transcriptionally active promoters. These 30 sequences can be amplified and re-cloned using the methods of the instant invention, or they can be rescued by virus packaging, reverse transcribed by the endogenous reverse

transcriptase reaction, and grown as plasmids (due to their plasmid origin of replication and the selectable kanamycin marker gene).

In addition to using a replication defective helper virus, such as the clone pPAM3, it is also possible to use a replication competent retrovirus, such as Moloney murine leukemia virus to passage the library. For use in human cells, however, the virus should have a tropism that is compatible with human cells (gibbon ape leukemia virus and amphotropic [4070A] murine retroviruses are acceptable).

In addition to being useful for generating active transcriptional promoters *de novo*, a small variation on the above procedures may enable the isolation of hormone responsive promoters. In it, the cells are treated with the hormone (which could be a steroid, a peptide hormone known to affect the cells, a drug, a drug agonist or antagonist, etc.) during passage. After isolation of surviving VL30 vector-containing cells, individual clones of drug resistant cells are tested for reporter gene expression with and without drug treatment to determine relative protein expression. Likewise, RNA expression can be determined by blot analysis or a similar method. A useful list of known VL30 responses to pharmacological agents is listed in Fig. 4.2 of Hodgson, 1996, *supra*, and can be used as a guide to help assess the potential agents known to have an effect on VL30 transcription.

Once the transcriptional promoters with the known specificity have been obtained, they can be used to obtain expression of genes from a variety of types of vectors. For example, in addition to retrovirus particles, the promoters can be incorporated into all other major groups of vectors: adenoviruses, herpes simplex virus vectors, DNA transfection vectors, etc. It will be apparent to persons of ordinary skill in the art that similar combinatorial libraries can also be used to screen for other characteristics than transcription activity in a particular cell. For example, combinatorial libraries of complementarity determining regions (CDRs) of antibodies or T cell receptors can be so screened using antibody screening methods, such as the phage display screening method (Pharmacia, Milwaukee, WI). Thus, the methods of this invention, particularly the combinatorial simplicity of this invention is a significant improvement over many *in vivo* recombination methods including those of (Stemmer, US Patent 5,605,793; 1997) that have described for the production of CDR combinatorial libraries.

**Example 5**  
**Gene Assembly Line**

5           From the above examples of 3 and 6 fragment gene self-assemblies, it is evident that assembly of genes by means of gene amplification, the use of offset restriction enzymes and incorporating unique, non-palindromic ends is a highly efficient process compared to conventional cloning methods. However, in addition to the considerations already discussed, it will be apparent to a person of ordinary skill in the art that the various  
10          procedures, protocols, methods and material of the instant invention become more difficult to use as the number of fragments increases. For example, if the efficiency of combining each fragment in an assemblage is 99%, then the overall efficiency of combining ten fragments will be 90%, the efficiency of combining 100 fragments will be 37%, etc. Therefore, a small drop in efficiency of any step or fragment, or a large increase in the complexity of the project,  
15          will be sufficient to reduce the overall efficiency. Fastidious procedures permit one to achieve success with more complex projects.

Foremost in its potential for inducing failure is human error in primer design where large numbers of fragments are used. Fortunately, the instant invention is suited to automation of most of the steps. This allows human input to be focused on design, analysis,  
20          and quality control. For the purposes of generating large vectors or chromosomes, it is desirable to provide an automated environment. One method to achieve this goal is a gene assembly line.

In a gene assembly line, multiple tasks are controlled by a machine or machines working together to increase speed and efficiency and to reduce human error. For  
25          example, computer aided design (CAD) and computer aided manufacturing (CAM) are incorporated and combined with the methods of this invention. The computers accept inputs in the form of template and primer sequences, together with preferences of regions to be copied and joined. The preferences include at least the sequences of the primer regions and information about the known restriction sites and maps of the sequences to be assembled, but  
30          ideally include the entire sequence. The preferences also include the number of sequences to be joined, the desired Tm for the primers, the list of potential restriction enzymes capable of offset digestion that are potential candidates for use in the assembly process, the desired end structures for each fragment terminus, a tag sequence (if any), whether circular or linear ends

are desired, and additional design considerations. The computer algorithm then searches the sequences to determine the candidate enzymes and specific primers that match the criteria of the input. Candidates for selection of unique non-palindromic overlaps are selected. The computer then posts selections or preferences for the type and order of end structures, the 5 primer binding sites, their potential for primer-dimer and intra-molecular interaction artifacts, and the potential conflicts with repeat sequences within the templates that could lead to incorrect polymerization. Based upon the selections made by the operator, the computer then determine the  $T_m$  for each primer, and makes adjustments (with suitable inputs from the investigator) to achieve a suitable  $T_m$  for the appropriate DNA synthesis or gene amplification 10 reaction. Ideally, the primers should have similar  $T_m$ 's so that all amplification reactions can be performed at once with one set of amplification instructions. In reality, it may be difficult to do this with complex projects. The output of this portion of the program, which can be in a generic format, such as a Microsoft Excel spreadsheet is then downloaded to a computerized oligonucleotide synthesizer, such as the Applied Biosystems 3928 nucleic acid synthesizer. 15 One advantage of using a computerized synthesizer is its robotic capability to de-protect and purify the oligonucleotides automatically. In addition this synthesizer can accept computerized input.

The quantity of individual oligos recovered is then determined spectrophotometrically. It is desirable to purify the oligonucleotides by high performance 20 liquid chromatography or by polyacrylamide gel. In a preferred embodiment, the oligonucleotides and templates are then assembled robotically using an automated nucleic acid handling system such as the Qiagen BioRobot 9600. The BioRobot is capable of accepting input from a computer and can combine the gene amplification reactions based upon the assignments of templates, primer and reagents provided in the input. The assembled 25 reactions are then amplified for example by PCR. In a preferred embodiment, the PCR heat block is incorporated into the robotic workspace and genes are assembled robotically but with minimal human intervention to change buffers, rearrange the platform, change programs, and the like. The resulting amplified products are also purified by the BioRobot or a similar robotic device. In a preferred embodiment, the robotic device uses Qiaquick cleanup 30 procedures, or a similar method and then assembles restriction endonuclease reactions to digest the purified gene amplification products. The gene amplification products are loaded onto a gel and electrophoresed. Human intervention may be necessary to analyze the

products and excise the correct fragments from the gel. At this point, the results are assessed and missing or incorrect sized fragments are resynthesized. The robotic device is preferably used to purify the gel fragments using Quiagen or similar cleanup procedures. After spectrophotometric quantitation of the purified fragments, the robotic device is preferably 5 used to assemble the ligation. Ideally the fragments are combined in an equimolar ratio of 1:1. However it is not necessary to use equimolar ratios in order to achieve gene self-assembly. For automated gene assembly, it may be desirable not to use equimolar ratios of input fragments, particularly if it simplified the task of quantitation. After ligation, the assemblies can be purified and ethanol precipitated or they can be added to the appropriate 10 host cells. Automation aids in maintaining the sterility of the reaction.

Several additional considerations can assist in the construction of long genes using gene assembly. First the number of fragments and the length of constructs are limiting factors. In addition to maintaining high standards of purify of both the oligonucleotide primers and gene amplification products, it is important to keep the error rate low during 15 copying. Thus, one can optimally start with 100 ng of template use only five rounds of gene amplification and finish with nearly 2 micrograms of product. This is more desirable for reducing errors than using a large number of amplification steps. It is also desirable to use a special copying enzyme such as *Pfu* DNA polymerase that has a low intrinsic error rate. Further it is desirable to use *in vivo* selection (in eukaryotic cells or tissues) rather than *E. coli* 20 cloning to reduce the incorporation of errors into the vectors. For example, a viral vector such as an adenoviral vector or the retro-vectors of the preceding examples are auto-selecting. A single correctly-assembled adenovirus vector molecule, for example, leads to a lytic infection (the viral products of which are cloned by limiting dilution on the appropriate eukaryotic cells), even though it may be combined in a ligation mix with a large excess of 25 incorrectly assembled molecules that are non-functional. Thus, it is not necessary to have a high efficiency, although high efficiency has been demonstrated in this system, in order to achieve success in making, for example gene therapy vectors.

For long fragments (3-30 kb), it is desirable to use enzymes and procedures that are designed or facilitate replication of long fragments, one such example is the 30 eLONGase system (Life Technologies). This system can copy up to 30 kb on a fragment with proofreading. Considerations for long PCR are reviewed in Beck, 1998. (The Scientist 6 January, 1998, pp. 16-18).

Internal restriction sites are a potential problem, particularly with large constructs and can be overcome in a number of ways. Use of alternate enzymes, methylation of internal restrictions sites (such as by using methylated DNA precursors during synthesis to leave the sites in primers unaffected, incorporation of the internal sites into the construct (if 5 they are non-palindromic), or mutagenesis of internal sites, are exemplary ways to deal with some of these issues.

For very large constructs, it is desirable to use enzymes such as *SapI* (recognizing 7 nucleotides and leaving a 3 bp overhang). This enzyme digests every 16,384 bp on average. There are 64 nucleotide triplet combinations, meaning that up to 32 fragments 10 can be ligated in a circle using *SapI*. *Fok1* and *Hga1* are other examples of class IIS enzymes that are useful for making large constructs. *Hga1* has 5 bp overhangs, permitting more than 500 *Hga1* fragments to be ligated. *Fok1* includes a Kozak ATG start codon. In a preferred embodiment, a *Fok1* site is inserted at the PuXXATG start site of a cDNA encoding 15 region. The cDNA is inserted in frame, providing a site for inserting and switching coding sequences within a vector.

It will be readily understood by those skilled in the art that the foregoing description has been for purposes of illustration only and that a variety of embodiments can be envisioned without departing from the scope of the invention. Therefore, it is intended 20 that the invention not be limited except by the claims.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

5

(i) APPLICANT: NATURE TECHNOLOGY CORPORATION, ET AL.

(ii) TITLE OF INVENTION: SELF-ASSEMBLING GENES, VECTORS AND USES THEREOF

10 (iii) NUMBER OF SEQUENCES: 68

## (iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: MUETING, RAASCH & GEBHARDT, P.A.
- (B) STREET: 119 NORTH FOURTH STREET, SUITE 203
- (C) CITY: MINNEAPOLIS
- (D) STATE: MINNESOTA
- (E) COUNTRY: USA
- (F) ZIP: 55401

20 (v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

25 (vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: Not Assigned
- (B) FILING DATE: 28-FEB-1998
- (C) CLASSIFICATION:

30 (vii) PRIORITY APPLICATION DATA:

- (A) APPLICATION NUMBER: 60/070,910
- (B) FILING DATE: 28-FEB-1997
- (C) CLASSIFICATION:

35 (viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: MCCORMACK, MYRA M.
- (B) REGISTRATION NUMBER: 36,602
- (C) REFERENCE/DOCKET NUMBER: 228.00010201

## (ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 612-305-1225
- (B) TELEFAX: 612-305-1228

45

## (2) INFORMATION FOR SEQ ID NO:1:

50 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6225 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: DNA (genomic)

60 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGAAGAATAA AAAATTACTG GCCTCTTG TG AGAACATGAA CTTTCACCTC GGAGCCCACC	60
CCCTCCCATC TCGAAAAC TC CAGTTATAAC TGGAGTTTT CCTTTAAAAG CTTGTGAAAA	120
65 ATTTGAGTCG TCGTCGAGAC TCCTCTACCC TGTGCAAAGG TGTATGAGTT TCGACCCAG	180

	AGCTCTGTGT GCTTTCTGTT GCTGCTTAT TTGACCCCCA GAGCTCTGGT CTGTGTGCTT	240
5	TCATGTCGCT GCTTTATTAA ATCTTACCTT CTACATTTA TGTATGGTCT CAGTGTCTTC	300
	TTGGGTACGC GGCTGTCCCG GGACTTGAGT GTCTGAGTGA GGGCTTCCC TCGAGGGTCT	360
	TTCATTGGT ACATGGGCCG GGAATTGGAG AATCTTCAT TTGGTGCATT GGCGGGAAAT	420
10	TCGAAAATCT TTCATTTGGT GCATTGGCCG GGAAACAGCG CGACCACCCA GAGGTCTTAG	480
	ACCCACTTAG AGGTAAGATT CTTTGTCTG TTTGGTCTG ATGTCTGTGT TCTGATGTCT	540
15	GTGTTCTGTT TCTAAGTCTG GTGCGATCGC AGTTTCAGTT TTGCGGACGC TCAGTGAGAC	600
	CGCGCTCCGA GAGGGAGTGC GGGGTGGATA AGGATAGACG TGTCCAGGTG TCCACCGTCC	660
	GTTCGCCCTG GGAGACGTCC CAGGAGGAAC AGGGGAGGAT CAGGGACGCC TGTTGGACCC	720
20	CTTTGAAGGC CAAGAGACCA TTTGGGTTG CGAGATCGTGGTTCAGTC CCACCTCGTGC	780
	CCCAGTTGCG AGATCGTGGG TTGAGTCACCTCGTGGTTCAGTC CCACCTCGTGC	840
25	CGAGTCCCAC CTCGCGTCTG GTCACGGGAT CGTGGGTTCG AGTCCCACCT CGTGTGTTGT	900
	TGCGAGATCG TGGGTTGAG TCCCACCTCG CGTCTGGTCA CGGGATCGTGGTCCACCT	960
	CCACCTCGTGCAGAGGGTCT CAATTGGCCG GCCTTAGAGA GGCCATCTGA TTCTTCTGGT	1020
30	TTCTCTTTT GTCTTAGTCT CGTGTCCGCT CTTGTTGTGA CTACTGTTT TCTAAAAATG	1080
	GGACAATCTG TGTCACACTCC CCTTTCTCTG ACTCTGGTTC TGTCGCTTGG TAATTTGTT	1140
35	TGTTTACGTT TGTTTTGAG AGTCGCTAT GTTGTCTGTT ACTATCTTGT TTTGTTGTTGT	1200
	GGTTTACGGT TTCTGTGTGT GTCTTGTGTGTCTCTGTTGAGTCAAGTTG GACTGATGAC	1260
	TGACGACTGT TTTTAAGTTA TGCCCTCTAA AATAAGCCTA AAAATCCTGT CAGATCCCTA	1320
40	TGCTGACCAC TTCCCTTCAG ATCAACAGCT GCCCTTACTC GAGCTCAAGC TTGAAATTCT	1380
	GCAGTCGACG GTACCGCGGC CGCTAACTAA TAGCCCATTCTCAAGGTAC GTAGGGGAA	1440
45	TCAATTCCGC CCCCCCCCTA ACGTTACTGG CCGAAGCCGC TTGGAATAAG GCCGGTGTGC	1500
	GTTTGTCTAT ATGTTATTTC CCACCATATT GCCGTCTTTT GGCAATGTGA GGGCCCGGAA	1560
	ACCTGGCCCT GTCTTCTTGA CGAGCATTCC TAGGGGTCTT TCCCCTCTCG CCAAAGGAAT	1620
50	GCAAGGTCTG TTGAATGTG TGAGGAAGC AGTCCCTCTG GAAGCTTCTT GAAGACAAAC	1680
	AACGTCTGTA GCGACCCCTT GCAGGCAGCG GAACCCCCCA CCTGGCGACA GGTGCCCTCG	1740
55	CGGCCAAAAG CCACGTGTAT AAGATACACC TGCAAAGGCG GCACAACCCC AGTGCCACGT	1800
	TGTGAGTTGG ATAGTTGTGG AAAGAGTCAA ATGGCTCTCC TCAAGCGTAT TCAACAAGGG	1860
	GCTGAAGGAT GCCCAGAAGG TACCCATTG TATGGGATCT GATCTGGGCG CTCGGTGCAC	1920
60	ATGCTTTACA TGTGTTTAGT CGAGGTTAAA AAAACGTCTA GGCCCCCGA ACCACGGGA	1980
	CGTGGTTTC CTTTGAAAAA CACGATACGG GATCCACCGG TCGCCACCAT GGGTAAAGGA	2040
65	GAAGAACTTT TCACAGGGAGT TGTCCCAATT CTTGTTGAAT TAGATGGTGA TGTTAATGGG	2100
	CACAAATTTT CTGTCAGTGG AGAGGGTGAA GGTGATGCAA CATACGGAAA ACTTACCCCTT	2160

	AAATTTATTT GCACTACTGG AAAACTACCT GTTCCATGGC CAACACTTGT CACTACTTC	2220
	ACTTATGGTG TTCAATGCTT TTCAAGATAC CCAGATCATA TGAAACGGCA TGACTTTTC	2280
5	AAGAGTGCCA TGCCCGAAGG TTATGTACAG GAAAGAACTA TATTTTCAA AGATGACGGG	2340
	AACTACAAGA CACGTGCTGA AGTCAAGTT GAAGGTGATA CCCTTGTAA TAGAATCGAG	2400
10	TTAAAAGGTA TTGATTTAA AGAAGATGGA AACATTCTG GACACAAATT GGAATACAAC	2460
	TATAACTCAC ACAATGTATA CATCATGGCA GACAAACAAA AGAATGGAAC CAAAGTTAAC	2520
	TCACAAAATTA GACACAAACAT TGAAGATGGA AGCGTTCAAC TAGCAGACCA TTATCAACAA	2580
15	AATACTCCAA TTGGCGATGG CCCTGTCCTT TTACCAGACA ACCATTACCT GTCCACACAA	2640
	TCTGCCCTT CGAAAGATCC CAACGAAAAG AGAGACCACA TGGTCCTCT TGAGTTGTA	2700
20	ACAGCTGCTG GGATTACACA TGGCATGGAT GAACTATACA AGTCCGGATC TAGATAACTG	2760
	TATCGATGGA TCCGAAGGCG GGGACAGCAG TGCAGTGGT GACAGAAAGC AAGTGATCTA	2820
	GGCCAGCAGC CTCCCTAAAG GGACTTCAGC CCACAAAGCC AAACTTGTGG CTTTAATACA	2880
25	AGCTCTGTAA ATGGTAAAAA AAAAAAAGTC TACACGGACA GCAGGTATGC TCTTGCCACT	2940
	GTACAGAGCA ATATAACAGAC AAAGAGAACT GTTGACATCT GCAGAGAAAG ACCTAAGATG	3000
30	CTGTGGCTAA AAGAAATCAG ATGGCAAATC TAACCGCCCA GGCATCCTAA AGAGCAATGA	3060
	TCCTGACAGT CTGAAGACTA TCAAGTTATA GACAAATTAA GACTGGTAAA AAAAAACCTG	3120
	TATAAAATAG TAAAAACTGA AAAAAGAAAA CTAGTCCTCT CATGAGAAGA CAGACCTGAC	3180
35	ATCTACTGAA AAATAGACTT TACTGGAAAA AATATGTGTA TGAATACCTT CTAGTTTTG	3240
	TGAACGTTCT CAAGATGGAT AAAAGCTTT CCTTGTAAAA CGAGACTGAT CAGATAGTCA	3300
	TCAAGAAGAT TGTTAAAGAA AATTTTCAA GGTTCGGAGT GCCAAAGCA ATAGTGTCA	3360
40	ATAATGGTCC TGCCTTTGTT GCCCAGGTAA GTCAGGGTGT GGCCAAGTAT TTAGAGGTCA	3420
	AATGAAAATT CCATTGTGTG TACAGACCTC AGAGCTCAGG AAAGATAAAA AAGAATAAAAT	3480
45	AAAACTCTAA ACAGACCTTG ACAAAATTAA TCCTAGAGAC TGGCACAGAC TTACTTGGTA	3540
	CTCCTTCCCC TTGCCCTATT TAGAACTGAG AATACTCCCT CTTGATTGG TTTTACTCTT	3600
50	TTTAAGATCC TTATGGGGC TCCTATGCCA TCACTGTCTT AAATGATGTG TTTAAACCTA	3660
	TGTTGTTATA ATAATGATCT ATATGTTAAG TTAAAAGGCT TGCAGGTGGT GCAGAAAGAA	3720
	GTCTGGTCAC AACTGGCTAC AGTGAACAAG CTGGGTACCC CAAGGACATC TTACCAAGTTC	3780
55	CAGCCAGAGA TCTGATCTAC GATCCCCGGG TCGACCCGGG TCGACCCCTGT GGAATGTGTG	3840
	TCAGTTAGGG TGTGGAAAGT CCCCAGGCTC CCCAGCAGGC AGAAGTATGC AAAGCATGCA	3900
60	TCTCAATTAG TCAGCAACCA GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT	3960
	GCAAAGCATG CATCTCAATT AGTCAGCAAC CATAGTCCCG CCCCTAACTC CGCCCATCCC	4020
	GCCCCCTAACT CCGCCCCAGTT CCGCCCCATTC TCCGCCCCAT GGCTGACTAA TTTTTTTTAT	4080
65	TTATGCAGAG GCCGAGGCCG CCTCGGCCTC TGAGCTATTG CAGAAGTAGT GAGGAGGCTT	4140
	TTTTGGAGGC CTAGGCTTTT GCAAAAAGCT TCACGCTGCC GCAAGCAGTC AGGGCGCAAG	4200

	GGCTGCTAAA GGAAGCGGAA CACGTAGAAA GCCAGTCCGC AGAACCGGTG CTGACCCCCG	4260
5	ATGAATGTCA GCTACTGGGC TATCTGGACA AGGGAAAACG CAAGCGCAA GAGAAAGCAG	4320
	GTAGCTTGCA GTGGGCTTAC ATGGCGATAG CTAGACTGGG CGGTTTTATG GACAGCAAGC	4380
	GAACCGGAAT TGCCAGCTGG GGCGCCCTCT GGTAAGGTTG GGAAGCCCTG CAAAGTAAAC	4440
10	TGGATGGCTT TCTTGCCGCC AAGGATCTGA TGGCGCAGGG GATCAAGATC TGATCAAGAG	4500
	ACAGGATGAG GATCGTTTCG CATGATTGAA CAAGATGGAT TGCACGCAGG TTCTCCGGCC	4560
15	GCTTGGGTGG AGAGGCTATT CGGCTATGAC TGGGCACAAC AGACAATCGG CTGCTCTGAT	4620
	GCCGCCGTGT TCCGGCTGTC AGCGCAGGGG CGCCCGGTT TC TTTTGTCAA GACCGACCTG	4680
	TCCGGTGCCC TGAATGAAC GCAGGACGAG GCAGCGCGC TATCGTGGCT GGCCACGACG	4740
20	GGCGTTCCCT GCGCAGCTGT GCTCGACGTT GTCACTGAAG CGGGAAAGGGG CTGGCTGCTA	4800
	TTGGGCGAAG TGCCGGGGCA GGATCTCTG TCATCTCACC TTGCTCCTGC CGAGAAAGTA	4860
25	TCCATCATGG CTGATGCAAT GCGGCGGCTG CATACTGTT ATCCGGCTAC CTGCCCATTC	4920
	GACCACCAAG CGAAACATCG CATCGAGCGA GCACGTACTC GGATGGAAGC CGGTCTTGT	4980
	GATCAGGATG ATCTGGACGA AGAGCATCAG GGGCTCGCGC CAGCCGAACCT GTTCGCCAGG	5040
30	CTCAAGGCAGC GCATGCCGA CGGCGAGGAT CTCGTCGTGA CCCATGGCGA TGCCCTGCTTG	5100
	CCGAATATCA TGGTGGAAAA TGGCCGCTTT TCTGGATTCA TCGACTGTGG CCGGCTGGGT	5160
35	GTGGCGGACC GCTATCAGGA CATAGCGTTG GCTACCCGTG ATATTGCTGA AGAGCTTGGC	5220
	GGCGAATGGG CTGACCGCTT CCTCGTGCTT TACGGTATCG CCGCTCCCGA TTCGCAGCGC	5280
	ATCGCCTTCT ATCGCCTTCT TGACGAGTTG TTCTGAGCGG GACTCTGGGG TTCGAAATGA	5340
40	CCGACCAAGC GACGCCAAC CTGCCATCAC GAGATTCGA TTCCACCGCC GCCTTCTATG	5400
	AAAGGTTGGG CTTCGGAATC GTTTTCCGGG ACGGAATTG TAATCTGCTG CTTGCAAACA	5460
	AAAAAACAC CGCTACCAGC GGTGGTTGT TTGCGGATC AAGAGCTACC AACTCTTTT	5520
45	CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA CTGTCCTTCT AGTGTAGCCG	5580
	TAGTTAGGCC ACCACTTCAA GAACTCTGTA GCACCGCCTA CATACTCGC TCTGCTAATC	5640
50	CTGTTACCAAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC TTACCGGGTT GGACTCAAGA	5700
	CGATAGTTAC CGGATAAGGC GCAGCGGTG GGCTGAACGG GGGGTTCGTG CACACAGCCC	5760
	AGCTTGGAGC GAACGACCTA CACCGAACTG AGATACCTAC AGCGTGAGCA TTGAGAAAGC	5820
55	GCCACGCTTC CCGAAGGGAG AAAGGCAGGAC AGGTATCCGG TAAGCGGCAG GGTCGGAACA	5880
	GGAGAGCGCA CGAGGGAGCT TCCAGGGGG AACGCCCTGGT ATCTTTATAG TCCTGTCGGG	5940
60	TTTCGCCACC TCTGACTTGA GCGTCGATTT TTGTGATGCT CGTCAGGGGG GCGGAGCCTA	6000
	TGGAAAAACG CCAGCAACGC CGAGATGCGC CGCCTCGAGT ACACCTGCGT CATGCTGAGA	6060
	CCCTCAAGCC TCACTAAAAG GGTCCCTGCC TAGTTCTGTT TACTAATCTG CCTTATTCTG	6120
65	TTTTTGTTC C CATGTTAAAG ATAGAGTAAA TGCAGTATTG TCCACATAGA GATATAGACT	6180

TCTGAAATTC TAAGATTAGA ATTATTTACA AGAAGAAGTG GGGAA

6225-

## (2) INFORMATION FOR SEQ ID NO:2:

- 5           (i) SEQUENCE CHARACTERISTICS:  
               (A) LENGTH: 487 base pairs  
               (B) TYPE: nucleic acid  
               (C) STRANDEDNESS: single  
               (D) TOPOLOGY: linear
- 10           (ii) MOLECULE TYPE: DNA (genomic)

15           (xii) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCTCCCATCT AGAGGTTGTT CTCGGAACAC TCCTAAACTT TTCACCCCAA AACTCCTCAC	60
20 CCTAAAGTTC GAAAAAAACTG TTCCAAGAAC ATTTTGAGA TAAAGGCCTC CTAGAACAAAC	120
CTCAAAATGA CATTGCCAAA TGATAAGACA TGACTCCTTA GTTACGTAGG TTCCCTTGATA	180
25 GGACATGACT CCTTAGTTAC GTAGGTTCCCT TGATAGGACA TGACTCCTTA GTTACGTAGA	240
TTCCCTTGTT AGAACTCCCT AGTGATGTA ACTTGTACTT TCCCTGCCCA GTTCTCCCCC	300
30 TTTGAGTTTT ACTATATAAG CCTGTAAAAA ATTTTGCTG ACCGTCGAGA CTCCTCTACC	360
CTGTGCTAAG GTGTATGAGT TTCGACCCCA GAGCTCTGTG TGCTTCCATG TTGCTGCTTT	420
45 ATTTCGACCC CAGAGCTCTG GTCTGTGTGC TTTCATGTGCT CGCTTTATT AAATCTTGCC	480
TTCTACAA	487

## (2) INFORMATION FOR SEQ ID NO:3:

- 40           (i) SEQUENCE CHARACTERISTICS:  
               (A) LENGTH: 366 base pairs  
               (B) TYPE: nucleic acid  
               (C) STRANDEDNESS: single  
               (D) TOPOLOGY: linear
- 45           (ii) MOLECULE TYPE: DNA (genomic)

50           (xiii) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCTCCCATCT AGAAAACATT TTTGAGATAA AGGCTTCCTG GAACAACCTC AAAATGAACC	60
AGGTACTCCT TAGTTACGTA GGTTCCCTGA TAGGACATGA CTCCTTAGTT ACATAGATT	120
55 CTTTGGCAGA ACTCCCTAGT GATGTAAACT TGTACTTTCC CTGCCAGTT CTCCCCCTTT	180
GAGTTTACT ATATAAGCCT GTGAAAAATT TTGGCTGACC GTCGAGACTC CTCTACCCCTG	240
60 TGCTAAGGTG TATGAGTTTC GACCCCAGAG CTCTGTGTGC TTCCATGTTG CTGCTTTATT	300
TCGACCCCAAG AGCTCTGGTC TGTGTGCTTT CATGTTGCTG CCTTATTAAA TCTTGCCCTC	360
TACATT	366

## (2) INFORMATION FOR SEQ ID NO:4:

- 65           (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 304 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

10 (xi) SEQUENCE DESCRIPTION: SEQ\_ID NO:4:

CCTCCCCATCT	AGAGATTGTT	CCCAGAACAC	TCCTGAACTC	TTCACCCCG	AATGCATGCC	60
TGAACTCCTC	ACCCTAGAGT	TCGAACCCCTC	CCAACTAAAG	ACTGTTCCAA	GAACATTTT	120
GAGATAAGGG	CCTCCTGGAA	CAACCTCAGA	ATGAACCGGG	TACATTGCCA	AATAATAGGA	180
CATGACCCCT	TAGTTACGTA	AAATCCCTTG	GCAGAACCCCC	TTGTCCCTTG	GCAGAACCCCC	240
TTAGTTATGT	AAACTTGTAC	TTTCCCTTACCC	CCGCTCTCCCC	CCCTTGAGTT	TTTCCTATAT	300
AAGC						304

25 (2) INFORMATION FOR SEO ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 304 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

40	CCTCCCCATCT AGAGAGTGTT CCCAGAACAC TCCTGAACTC TTCACCCAG AATGCATTCC TGAACCTCCTC ACCCTAGAGT TCGAACCTCT CCAACTAAAG ACTGTTCCAA GAACATTTTT	60 120
45	GAGATAAGGG CCTCCTGGAA CAACCTCAGA ATGAACCGGA TACATTGCCA AATAATAGGA CATGACCCCT TAGTTACGTA GAATCCCTTG GCAGAACCCCC TTGTCCCTTG GCAGAACCCCC	180 240
	TTAGTTATGT AAACTTGTAC TTTCCCTTACC CCGCTCTCCC CCCTTGAGTT TTTCCCTATAT	300
50	AAGC	304

(2) INFORMATION FOR SEQ ID NO:6:

55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 304 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

63 CCTCCCCATCT AGAGAGTGTG CCCAGAACAC TCCTGAACTC TTCAACCCAG AATGCATTCC 60

TGAACTCCTC ATCCTAGAGT TCGAACCTC CCAACTAAAG ACTGTTCAA GAACATTTT  
 5 GAGATAAGGG CCTCCTGGAA CAACCTCAGA ATGAACCTGG TACATTGCCA AATAATAGGA  
 CATGACCCTT TAGTTACGTA GAATCCCTTG GCAGAACCCC TTGTCCCTTG GCAGAACCCC  
 TTAGTTATGC AAACTTGTAC TTTCTCTGCC CCGCTCTCCC CCCTTGAGTT TTTCTATAT  
 10 AAGC 304

## (2) INFORMATION FOR SEQ ID NO:7:

15 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 304 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: DNA (genomic)

## 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCTCCCATCT AGAGAGTGTT CCCAGAACAC TCCTGAACTC TTCACCTCAA AATGCATTCC  
 30 GAGATAAGGG CCTCCTGGAA CAACCTCAGA ATGAACCCAG TACATTGCCA AATAATAGGA  
 CATGACCCTT TAGTTACGTA GAATCCCTTG GCAGAACCCC TTGTCCCTTG GCAGAACCCC  
 35 TTAGTTATGC AAACTTGTAC TTTCTCTGCC CCGCTCTCCC CCCTTGAGTT TTTCTATAT  
 AAGC 304

## 40 (2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 305 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 45 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCTCCCATCT AGAGATTGTT CCCAGAACAC TCCTGAACTC TTCACCCAG AATGCATTCC  
 55 GAGATAAGGG CCTCCTGGAA CAACCTCAGA ATGAACCGGA TACATTGCCA AATAATAGGA  
 60 CATGACCCTT TAGTTACGTA GAATTCCCTT GGCAAGAACCC CTTGTCCCTT GGCAAGAACCC  
 CTTAGTTATG CAAACTTGTAC CTTCCTGTC CCCGCTCTCC CCCCTTGAGG TTTCTATA  
 TAAGC 305

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:

  - (A) LENGTH: 305 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

10

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

15	CCTCCCCATCT AGAGAGTGTT CCCAGAACAC TCCTGAACTC TTCACCCAG AATGCATTCC	60
	TGAACCCCTC ACCCTAGAGT TCGAACCCCTC CCAACTAAAG ACTGTTCCAA GAACATTTTT	120
20	GAGATAAGGG CCTCCTGGAA CAACCTCAGA ATGAACCAGG TACATTGCCA ATAATAGGA	180
	CATGACCCCT TAGTTACGTA GAATTCCCTT GGCAGAACCC CTTGTCCCTT GGCAGAACCC	240
	CTTAGTTATG CGAACTTGT A CTTCCCTGC CCCGCTCTCC CCCCTTGAGT TTTTCCTATA	300
25	TAAGC	305

- (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:

  - (A) LENGTH: 306 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: DNA (genomic)

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCCTCCCATC TAGAGAGTGT TCCCAGAAC A CTCCTGAAC T CTTCATCCC A GAATGCATT C	60
CTGAACTCCT CACCCTATAG TT CGAACCCCT CCCAACTAAA GACTGTTCCA AGAACATTT	120
TGAGATAAGG GCCTCCCTGG A ACAACCTCG A ATGAAACCGG GTACATTGCC AAATAATAGG	180
ACATGACCCC TTAGTTACGT AGAATTCCCT TGGCAGAAC C CCTTGTCGCT TGGCAGAAC C	240
CCTTAGTTAT GTAAACTTGT ACTTTCCCTG CCCCAGCTCT C CCCCCCTTGAG TTTTTACTAT	300
<b>ATAAGC</b>	306

- (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:

  - (A) LENGTH: 305 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

65

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCTCCCCATCT	AGAGAGTGTT	CCCAAAACAC	TCCTGAACTC	TTCACCCAG	AATGCATTCC	60	
5	TGAACTCCTC	ACCCTAAAGT	TCAAACCCCTC	CCAACTAAAG	ACTGTTCCAA	GAACATTTTT	120
	GAGATAAGGG	CCTCCTGGAA	CAACCTCAGA	ATGAACCGGG	TACATTGCCA	AATAATAGGA	180
10	CATGACCCCT	TAGTTACACA	GAATTCCCTT	GGCAAAACCC	CTTGTCCCTT	GGCAGAACCC	240
	CTTAGTTATG	CAAACTTGTA	CTTTCCCTGC	CCAGCTCTCC	CCCCTTGAGT	TTTCCTATA	300
	TAAGC						305

## 15 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 304 base pairs  
 (B) TYPE: nucleic acid  
 20 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

25

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCTCCCCATCT	AGAGAGTGTT	CCCAGAACAC	TCCTGAACTC	TTCACCCAG	AATGCATTCC	60	
30	TGAACTCCTC	ACCCTAGAGT	TTGAACCCCTC	CCAACTAAAG	ACTGTTCCAA	GAACATCTTT	120
	GAGATAAGGG	CCTCCTGGAA	CAACCTCAGA	ATGAACCGGG	TACATTGCCA	AATAATAGGA	180
35	CATGACCCCT	TAGTTACGTA	GAATTCCCTT	GGCAGAACCC	CTTGTCGCTT	GGCAGAACCC	240
	CTTAGTTATG	CAAACTTGTA	CTTTCCCTGC	CCCGCTCTCC	CCCTTGAGTT	TTTCCTATAT	300
40	AAGC						304

## 40 (2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 303 base pairs  
 (B) TYPE: nucleic acid  
 45 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: DNA (genomic)

## 55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CCTCCCCATCT	AGAGAGTGTT	CCCAGAACAC	TCCTAAACTC	TTCACCCAG	AATGCATTCC	60	
	TGAACTCCTC	ACCCTAGAGT	TCGAACCCCT	CCAACTAAAG	ACTGTTCCAA	GAACATTTTT	120
60	GAGATAAGGG	CCTCCTGGAA	CAACCTCAAA	ATGAACCGGG	TACATTGCCA	AATGATAGGA	180
	CATGACCCCT	TAGTTACGTA	GATTCCCTTG	GCAGAACCCCC	TTGTCCCTTG	GCAGAACCCCC	240
65	CTAGTGATGT	AAACTTGTAC	TTTCCCTGCC	CAGCTCTCCC	CCCTTGAGTT	TTCCTATATA	300
	AGC						303

## (2) INFORMATION FOR SEQ ID NO:14:

5                   (i) SEQUENCE CHARACTERISTICS:  
                   (A) LENGTH: 8657 base pairs  
                   (B) TYPE: nucleic acid  
                   (C) STRANDEDNESS: single  
                   (D) TOPOLOGY: linear

10               (ii) MOLECULE TYPE: DNA (genomic)

15               (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TGAAGAATAA	AAAATTACTG	GCCTCTTGTG	AGAACATGAA	CTTCACCTC	GGAGCCCACC	60
CCCTCCCATC	TGGAAAACAT	ACTTGAGAAA	AACATTTCT	GGAACAACCA	CAGAATGTTT	120
20              CAACAGGCCA	GATGTATTGC	CAAACACAGG	ATATGACTCT	TTGGTTGAGT	AAATTGTGG	180
TTGTTAAACT	TCCCCTATT	CCTCCCCATT	CCCCCTCCCA	GTGGTGGTT	TTTCCTTTA	240
25              AAAGCTTGTG	AAAAATTGA	GTCGTCGTCG	AGACTCCTCT	ACCCGTGCA	AAGGTGTATG	300
AGTTTCGACC	CCAGAGCTCT	GTGTGCTTTC	TGTTGCTGCT	TTATTCGAC	CCCAGAGCTC	360
30              TGGTCTGTGT	GCTTTCATGT	CGCTGCTTTA	TTAAATCTTA	CCTTCTACAT	TTTATGTATG	420
GTCTCAGTGT	CTTCTTGGGT	ACCGCGCTGT	CCCCGGACTT	GAGTGTCTGA	GTGAGGGTCT	480
35              TCCCTCGAGG	GTCTTCATT	TGGTACATGG	GCCGGGAATT	CGAGAATCTT	TCATTGGTG	540
CATTGGCCGG	GAATTCGAAA	ATCTTCATT	TGGTGCATTG	GCCGGGAAAC	AGCGCGACCA	600
40              CCCAGAGGTC	CTAGACCCAC	TTAGAGGTAA	GATTCTTGT	TCTGTTTGG	TCTGATGTCT	660
GTGTTCTGAT	GTCTGTGTT	TGTTCTAAG	TCTGGTGCAG	TCGCAGTTTC	AGTTTGCAG	720
45              ACGCTCAGTG	AGACCGCGCT	CCGAGAGGGA	GTGCGGGGTG	GATAAGGATA	GACGTGTCCA	780
GGTGTCCACC	GTCCGTTCGC	CCTGGGAGAC	GTCCCAGGAG	GAACAGGGGA	GGATCAGGGGA	840
50              CGCCTGGTGG	ACCCCTTTGA	AGGCCAAGAG	ACCATTGGG	GTTGCGAGAT	CGTGGGTTCG	900
AGTCCCACCT	CGTGCCCAGT	TGCGAGATCG	TGGGTTCGAG	TCCCACCTCG	TGTTTGTG	960
55              CGAGATCGTG	GGTTCGAGTC	CCACCTCGCG	TCTGGTCACG	GGATCGTGGG	TTCGAGTCCC	1020
ACCTCGTGT	TTGTTGCGAG	ATCGTGGTT	CGAGTCCCAC	CTCGCGTCTG	GTCACGGGAT	1080
60              CGTGGGTTCG	AGTCCCACCT	CGTGCAGAGG	GTCTCAATTG	GCCGGCCTTA	GAGAGGCCAT	1140
CTGATTCTTC	TGGTTCTCT	TTTGTCCTTA	GTCTCGTGT	CGCTCTTGT	GTGACTACTG	1200
TTTTCTAAA	AATGGGACAA	TCTGTGTCCA	CTCCCCTTTC	TCTGACTCTG	GTTCTGTCG	1260
65              TTGGTAATT	TGTTGTTTA	CGTTGTTTT	TGTGAGTCGT	CTATGTTGTC	TGTTACTATC	1320
TTGTTTTGT	TTGTGGTTA	CGGTTCTGT	GTGTGCTTG	TGTGTCCTT	TGTGTCAGA	1380
CTTGGACTGA	TGACTGACGA	CTGTTTTAA	GTTATGCCCT	CTAAAATAAG	CCTAAAAATC	1440
CTGTCAGATC	CCTATGCTGA	CCACTTCCTT	TCAGATCAAC	AGCTGCCCTT	ACGTATCGAT	1500

	GGATCCCTCG ACTAACTAAT AGCCCATTCT CCAAGGTCGA GCGGGATCAA TTCCGCC	1560
	CCCCCTAACGT TACTGGCCGA AGCCGCTTGG AATAAGGCCG GTGTGCGTTT GTCTATATGT	1620
5	TATTTTCCAC CATATTGCCG TCTTTGGCA ATGTGAGGGC CCGGAAACCT GGCCCTGTCT	1680
	TCTTGACGAG CATTCCCTAGG GGTCTTCCC CTCTGCCAA AGGAATGCAA GGTCTGTTGA	1740
10	ATGTCGTGAA GGAAGCAGTT CCTCTGGAAG CTTCTTGAAG ACAAAACAACG TCTGTAGCGA	1800
	CCCTTGCAG GCAGCGAAC CCCCCACCTG GCGACAGGTG CCTCTGCGC CAAAAGCCAC	1860
	GTGTATAAGA TACACCTGCA AAGGCGGCAC AACCCCAGTG CCACGTTGTG AGTTGGATAG	1920
15	TTGTGGAAAG AGTCAAATGG CTCTCCTCAA GCGTATTCAA CAAGGGGCTG AAGGATGCC	1980
	AGAAGGTACC CCATTGTATG GGATCTGATC TGCGCACATGC TTTACATGTG	2040
20	TTTAGTCGAG GTTAAAAAAA CGTCTAGGCC CCCCGAACCA CGGGGACGTG GTTTCCCTT	2100
	GAAAAACACG ATAATAATCA TGGGCGCGGA TCCCCTCGTT TTACAACGTC GTGACTGGGA	2160
	AAACCCCTGGC GTTACCCAAC TTAATCGCCT TGCAACAT CCCCCCTTCG CCAGCTGGCG	2220
25	TAATAGCGAA GAGGCCCGCA CCGATGCC CTTCCAAACAG TTGCGCAGCC TGAATGGCGA	2280
	ATGGCGCTTT GCCTGGTTTC CGGCACCAGA AGCGGTGCCG GAAAGCTGGC TGGAGTGC	2340
30	TCTTCCTGAG GCCGATACTG TCGTCGTCCC CTCAAACCTGG CAGATGCACG GTTACGATGC	2400
	GCCCCATCTAC ACCAACGTA CCTATCCCAT TACGGTCAAT CCGCCGTTTG TTCCCACGGA	2460
	GAATCCGACG GGGTGTACT CGCTCACATT TAATGTTGAT GAAAGCTGGC TACAGGAAGG	2520
35	CCAGACGCGA ATTATTTTG ATGGCGTTAA CTCGGCGTTT CATCTGTGGT GCAACGGCG	2580
	CTGGGTCGGT TACGGCCAGG ACAGTCGTTT GCCGCTGAA TTTGACCTGA GCGCATTTTT	2640
40	ACGCGCCGGA GAAAACCGCC TCGCGGTGAT GGTGCTGCGT TGGAGTGACG GCAGTTATCT	2700
	GGAAGATCAG GATATGTGGC GGATGACCGG CATTTCCTG GACGTCTCGT TGCTGCATAA	2760
	ACCGACTACA CAAATCAGCG ATTTCCATGT TGCCACTCGC TTTAATGATG ATTCAGCCG	2820
45	CGCTGTACTG GAGGCTGAAG TTCAGATGTG CGGCGAGTTG CGTACTACC TACGGTAAC	2880
	AGTTTCTTA TGGCAGGGTG AAACGCAGGT CGCCAGCGC ACCGCGCCTT TCAGCGGTGA	2940
50	AATTATCGAT GAGCGTGGTG GTTATGCCGA TCGCGTCACA CTACGTCTGA ACGTCGAAA	3000
	CCCGAAACTG TGGAGCGCCG AAATCCGAA TCTCTATCGT GCGGTGGTTG AACTGCACAC	3060
	CGCCGACGGC ACGCTGATTG AAGCAGAACG CTGCGATGTC GGTTCCGCG AGGTGCGGAT	3120
55	TGAAAATGGT CTGCTGCTGC TGAACGGCAA GCCGTTGCTG ATTGAGGGCG TTAACCGTCA	3180
	CGAGCATCAT CCTCTGCATG GTCAGGTCA GGATGAGCAG ACGATGGTC AGGATATCCT	3240
	GCTGATGAAG CAGAACAACT TTAACGCCGT GCGCTGTTCG CATTATCCGA ACCATCCGCT	3300
60	GTGGTACACG CTGTGCGACC GCTACGGCCT GTATGTTGTG GATGAAGCCA ATATTGAAAC	3360
	CCACGGCATG GTGCCAATGA ATCGTCTGAC CGATGATCCG CGCTGGCTAC CGGCGATGAG	3420
	CGAACCGCGTA ACGCGAATGG TGCAGCGCGA TCGTAATCAC CCGAGTGTGA TCATCTGGTC	3480
65	GCTGGGAAAT GAATCAGGCC ACGGCCTAA TCACGACGCG CTGTATCGCT GGATCAAATC	3540

	TGTCGATCCT	TCCCGCCCCG	TGCAGTATGA	AGGC GGCGGA	GCCGACACCA	CGGCCACCAGA	3600
5	TATTATTTGC	CCGATGTACG	CGCGCGTGG	TGAAGACCAG	CCCTTCCCGG	CTGTGCCGAA	3660
	ATGGTCCATC	AAAAAAATGGC	TTTCGCTACC	TGGAGAGACG	CGCCCGCTGA	TCCTTGCGA	3720
	ATACGCCAAC	GCGATGGGTA	ACAGTCTTGG	CGGTTTCGCT	AAATACTGGC	AGGC GTTTCG	3780
10	TCAGTATCCC	CGTTTACAGG	GCGGCTTCGT	CTGGGACTGG	GTGGATCAGT	CGCTGATTAA	3840
	ATATGATGAA	AACGGCAACC	CGTGGTCGGC	TTACGGCGGT	GATTTGGCG	ATACGCCGAA	3900
15	CGATGCCAG	TTCTGTATGA	ACGGTCTGGT	CTTTGCCGAC	CGCACGCCGC	ATCCAGCGCT	3960
	GACGGAAGCA	AAACACCAGC	AGCAGTTTTT	CCAGTTCCGT	TTATCCGGGC	AAACCATCGA	4020
	AGTGACCAGC	GAATACCTGT	TCCGTATAG	CGATAACGAG	CTCCTGCACT	GGATGGTGGC	4080
20	GCTGGATGGT	AAGCCGCTGG	CAAGCGGTGA	AGTGCCTCTG	GATGTCGCTC	CACAAGGTAA	4140
	ACAGTTGATT	GAAC TGCCCTG	AACTACCGCA	GCCGGAGAGC	GCCGGGCAAC	TCTGGCTCAC	4200
25	AGTACCGCGTA	GTGCAACCGA	ACGCGACCGC	ATGGTCAGAA	GCCGGGCACA	TCAGGCCCTG	4260
	GCAGCAGTGG	CGTCTGGCGG	AAAACCTCAG	TGTGACGCTC	CCC GCCGCGT	CCCAGGCCAT	4320
	CCCGCATCTG	ACCACCA CGCG	AAATGGATTT	TTGCATCGAG	CTGGGTAATA	AGCGTTGGCA	4380
30	ATTTAACCGC	CAGTCAGGCT	TTCTTCACA	GATGTTGATT	GGCGATAAAA	AACA ACTGCT	4440
	GACGCCGCTG	CGCGATCAGT	TCACCCGTGC	ACCGCTGGAT	AAACGACATTG	GC G T A A G T G A	4500
35	AGCGACCCGC	ATTGACCCCTA	ACGCCTGGGT	CGAACGCTGG	AAAGCGGGGG	GCCATTACCA	4560
	GGCCGAAGCA	GC G T T G T T G C	AGTGCACGGC	AGATACACTT	GCTGATGGGG	TGCTGATTAC	4620
	GACCGCTCAC	GC G T G G C A G C	ATCAGGGGAA	AACCTTATTT	ATCAGCCGGA	AAACCTACCG	4680
40	GATTGATGGT	AGTGGTCAAA	TGGCGATTAC	CGTTGATGTT	GAAGTGGCGA	GCGATACACC	4740
	GCATCCGGCG	CGGATTGGCC	TGA ACTGCCA	GCTGGCGCAG	GTAGCAGAGC	GGGTAAACTG	4800
45	GCTCGGATTA	GGGCCGCAAG	AAA ACTATCC	CGACCGCCTT	ACTGCCGCCT	GT T T T G A C C G	4860
	CTGGGATCTG	CCATTGTCAG	ACATGTATAC	CCCGTACGTC	TTCCCGAGCG	AAA ACGGTCT	4920
	GCGCTGCGGG	ACCGCGGAAT	TGA ATTATGG	CCCACACCAG	TGGCGGGCG	ACTTCCAGTT	4980
50	CAACATCAGC	CGCTACAGTC	AACAGCAACT	GATGGAAACC	AGCCATCGCC	ATCTGCTGCA	5040
	CGCGGAAGAA	GGCACATGGC	TGA ATATCGA	CGGTTCCAT	ATGGGGATTG	GTGGCGACGA	5100
55	CTCCTGGAGC	CCGTCAGTAT	CGCGGAATT	CCAGCTGAGC	GCCGGTCGCT	ACCATTACCA	5160
	GTTGGTCTGG	TGTCAAAAT	AAATAATAACC	GGGCAGGGGG	GATCCGAAGG	CGGGGACAGC	5220
	AGTGCAGTGG	TGGACAGAAA	GCAAGTGATC	TAGGCCAGCA	GCCTCCCTAA	AGGGACTTCA	5280
60	GCCCACAAAG	CCAAACTTGT	GGCTTAATA	CAAGCTCTGT	AAATGGTAAA	AAAAAAAAAG	5340
	TCTACACGGA	CAGCAGGTAT	GCTCTGCCA	CTGTACAGAG	CAATATACAG	ACAAAGAGAA	5400
	CTGTTGACAT	CTGCAGAGAA	AGACCTAAGA	TGCTGTTGGCT	AAAAGAAATC	AGATGGCAA	5460
65	TCTAACCGCC	CAGGCATCCT	AAAGAGCAAT	GATCCTGACA	GTCTGAAGAC	TATCAAGTTA	5520

	TAGACAAATT AAGACTGGTA AAAAAAAACCC TGTATAAAAT AGTAAAAACT GAAAAAAAGAA	5580
	AACTAGTCCT CTCATGAGAA GACAGACCTG ACATCTACTG AAAAATAGAC TTTACTGGAA	5640
5	AAAATATGTG TATGAATACC TTCTAGTTT TGTGAACGTT CTCAAGATGG ATAAAAGCTT	5700
	TTCCCTGTAA AACGAGACTG ATCAGATAGT CATCAAGAAG ATTGTTAAAG AAAATTTCC	5760
10	AAGGTTCGGA GTGCCAAAAG CAATAGTGTC AGATAATGGT CCTGCCTTG TTGCCAGGT	5820
	AAGTCAGGGT GTGGCCAAGT ATTTAGAGGT CAAATGAAAA TTCCATTGTG TGTACAGACC	5880
	TCAGAGCTCA GGAAAGATAA AAAAGAATAA ATAAAACCT AAACAGACCT TGACAAAATT	5940
15	AATCCTAGAG ACTGGCACAG ACTTACTTGG TACTCCTTCC CTTGCCCTA TTTAGAACTG	6000
	AGAATACTCC CTCTTGATTG GGTTTTACTC TTTTTAAGAT CTTTATGGG GCTCCTATGC	6060
20	CATCACTGTC TTAAATGATG TGTAAACCT TATGGTGTG TAATAATGAT CTATATGTTA	6120
	AGTTAAAAGG CTTGCAGGTG GTGCAGAAAG AAGTCTGGTC ACAACTGGCT ACAGTGAACA	6180
	AGCTGGGTAC CCCAAGGACA TCTTACCAAGT TCCAGCCAGA GATCTGATCT ACGATCCCCG	6240
25	GGTCGACCCG GGTCGACCCCT GTGGAATGTG TGTCAGTTAG GGTGTGGAAA GTCCCCAGGC	6300
	TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC CAGGTGTGGA	6360
30	AAGTCCCCAG GCTCCCCAGC AGGCAGAAAGT ATGCAAAGCA TGCATCTCAA TTAGTCAGCA	6420
	ACCATAGTCC CGCCCCAAC TCCGCCATC CCGCCCCCTAA CTCCGCCAG TTCCGCCAT	6480
	TCTCCGCCAG ATGGCTGACT AATTTTTTT ATTATGCAG AGGCCGAGGC CGCCTCGGCC	6540
35	TCTGAGCTAT TCCAGAAGTA GTGAGGAGGC TTTTTGGAG GCCTAGGCTT TTGCAAAAG	6600
	CTTCACGCTG CCGCAAGCAC TCAGGGCGCA AGGGCTGCTA AAGGAAGCGG AACACGTAGA	6660
40	AAGCCAGTCC GCAGAACCGG TGCTGACCCC GGATGAATGT CAGCTACTGG GCTATCTGGA	6720
	CAAGGGAAAA CGCAAGCGCA AAGAGAAAGC AGGTAGCTT CAGTGGCTT ACATGGCGAT	6780
	AGCTAGACTG GCGGGTTTTA TGGACAGCAA GCGAACCGGA ATTGCCAGCT GGGGCCCT	6840
45	CTGGTAAGGT TGGGAAGCCC TGCAAAGTAA ACTGGATGGC TTTCTGCCG CCAAGGATCT	6900
	GATGGCGCAG GGGATCAAGA TCTGATCAAG AGACAGGATG AGGATCGTT CGCATGATTG	6960
50	AACAAGATGG ATTGCACGCA GGTTCTCCGG CCGCTTGGGT GGAGAGGCTA TTCGGCTATG	7020
	ACTGGCACA ACAGACAATC GGCTGCTCTG ATGCCGCCGT GTTCCGGCTG TCAGGGCAGG	7080
	GGCGCCCGGT TCTTTTGTC AAGACCGACC TGTCCGGTGC CCTGAATGAA CTGCAGGACG	7140
55	AGGCAGCGCG GCTATCGTGG CTGGCCACGA CGGGCGTTCC TTGCGCAGCT GTGCTCGACG	7200
	TTGTCACTGA AGCGGGAGG GACTGGCTGC TATTGGCGA AGTGGCGGG CAGGATCTCC	7260
	TGTCATCTCA CCTTGCTCCT GCCGAGAAAG TATCCATCAT GGCTGATGCA ATGCCGGGC	7320
60	TGCATACGCT TGATCCGGCT ACCTGCCCAT TCGACCACCA AGCGAAACAT CGCATCGAGC	7380
	GAGCACGTAC TCGGATGGAA GCCGGTCTTG TCGATCAGGA TGATCTGGAC GAAGAGCCTC	7440
65	AGGGGCTCGC GCCAGCCGAA CTGTTGCCA GGCTCAAGGC GCGCATGCC GACGGCGAGG	7500
	ATCTCGTCGT GACCCATGGC GATGCCCTGCT TGCCGAATAT CATGGTGGAA AATGGCCGCT	7560

	TTTCTGGATT CATCGACTGT GGCCGGCTGG GTGTGGCGGA CCGCTATCAG GACATAGCGT	7620
5	TGGCTACCCG TGATATTGCT GAAGAGCTTG GCAGCGAATG GGCTGACCGC TTCTCGTGC	7680
	TTTACGGTAT CGCCGCTCCC GATTCGCAGC GCATCGCCTT CTATCGCCTT CTTGACGAGT	7740
	TCTTCTGAGC GGGACTCTGG GGTCGAAAT GACCGACCAA GCGACGCCA ACCTGCCATC	7800
10	ACGAGATTC GATTCCACCG CCGCCTCTA TGAAAGGTTG GGCTTCGGAA TCGTTTCCG	7860
	GGACGGAATT CGTAATCTGC TGCTTGCAAA CAAAAAAACC ACCGCTACCA GCGGTGGTT	7920
15	GTTTGCCTGA TCAAGAGCTA CCAACTCTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC	7980
	AGATAACAAA TACTGTCCTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAACTCTG	8040
	TAGCACCGCC TACATACCTC GCTCTGCTAA TCCTGTTACC AGTGGCTGCT GCCAGTGGCG	8100
20	ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG GCGCAGCGGT	8160
	CGGGCTGAAC GGGGGGTTCG TGCACACAGC CCAGCTTGGG GCGAACGACC TACACCGAAC	8220
25	TGAGATACCT ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCCGAAGGG AGAAAGGCAG	8280
	ACAGGTATCC GGTAAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG	8340
	GAAACGCCCTG GTATCTTAT AGTCCTGTCG GGTTTCGCCA CCTCTGACTT GAGCGTCGAT	8400
30	TTTTGTGATG CTCGTCAGGG GGGCGGAGCC TATGGAAAAA CGCCAGCAAC GCCGAGATGC	8460
	GCCGCCCTCGA GTACACCTGC GTCATGCTGA GACCCTCAAG CCTCACTAAA AGGGTCCCTG	8520
35	CCTAGTTCTG TTTACTAATC TGCCTTATTC TGTTTTGTT CCCATGTTAA AGATAGAGTA	8580
	AATGCAGTAT TCTCCACATA GAGATATAGA CTTCTGAAAT TCTAAGATTA GAATTATTTA	8640
	CAAGAAGAAG TGGGGAA	8657

## 40 (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6359 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

50

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

55	TGAAGAATAA AAAATTACTG GCCTCTTGTG AGAACATGAA CTTTCACCTC GGAGCCCACC	60
	CCCTCCCATC TGAAAACAT ACTTGAGAAA AACATTTCT GGAACAACCA CAGAATGTTT	120
60	CAACAGGCCA GATGTATTGC CAAACACAGG ATATGACTCT TTGGTTGAGT AAATTGTGG	180
	TTGTTAAACT TCCCCTATTC CCTCCCCATT CCCCTCCCA GTTTGTGGTT TTTCTTTA	240
	AAAGCTTGTG AAAAATTGA GTCGTGTCG AGACTCCTCT ACCCTGTGCA AAGGTGTATG	300
65	AGTTTCGACC CCAGAGCTCT GTGTGCTTTC TGTTGCTGCT TTATTCGAC CCCAGAGCTC	360

	TGGTCTGTGT	GCTTTCATGT	CGCTGCTTTA	TTAAATCTTA	CCTTCTACAT	TTTATGTATG	420
	GTCTCAGTGT	CTTCTTGGGT	ACGCGGCTGT	CCCGGGACTT	GAGTGTCTGA	GTGAGGGTCT	480
5	TCCCTCGAGG	GTCTTCATT	TGGTACATGG	GCCGGGAATT	CGAGAACATT	TCATTGGTG	540
	CATTGCCCG	GAATTCGAAA	ATCTTCATT	TGGTGCATTG	GCCGGGAAAC	AGCGCGACCA	600
10	CCCAGAGGTC	CTAGACCCAC	TTAGAGGTA	GATTCTTGT	TCTGTTTGG	TCTGATGTCT	660
	GTGTTCTGAT	GTCTGTGTT	TGTTCTAAG	TCTGGTGCGA	TCGCAGTTTC	AGTTTGCGG	720
	ACGCTCAGTG	AGACCGCGCT	CCGAGAGGGA	GTGCGGGGTG	GATAAGGATA	GACGTGTCCA	780
15	GGTGTCCACC	GTCCGTTCGC	CCTGGGAGAC	GTCCCAGGAG	GAACAGGGGA	GGATCAGGGA	840
	CGCCTGGTGG	ACCCCTTGA	AGGCCAAGAG	ACCATTGGG	GTTGCGAGAT	CGTGGGTTCG	900
20	AGTCCCACCT	CGTGCCCCAGT	TGCGAGATCG	TGGGTTCGAG	TCCCACCTCG	TGTTTGTG	960
	CGAGATCGTG	GGTCGAGTC	CCACCTCGCG	TCTGGTCACG	GGATCGTGGG	TTCGAGTCCC	1020
	ACCTCGTGT	TTGTTGCGAG	ATCGTGGTT	CGAGTCCCAC	CTCGCGTCTG	GTCACGGGAT	1080
25	CGTGGGTTCG	AGTCCCACCT	CGTGCAGGAG	GTCTCAATTG	GCCGGCCTTA	GAGAGGCCAT	1140
	CTGATTCTTC	TGTTTCTCT	TTTGTCTTA	GTCTCGTGT	CGCTCTTGT	GTGACTACTG	1200
30	TTTTCTAAA	AATGGGACAA	TCTGTGTCCA	CTCCCCTTTC	TCTGACTCTG	GTTCTGTGCG	1260
	TTGGTAATTT	TGTTGTTTA	CGTTTGTGTT	TGTGAGTCGT	CTATGTTGTC	TGTTACTATC	1320
	TTGTTTTGT	TTGTGGTTA	CGGTTCTGT	GTGTGTCTTG	TGTGTCTCTT	TGTGTTCAGA	1380
35	CTTGGACTGA	TGACTGACGA	CTGTTTTAA	GTTATGCCTT	CTAAAATAAG	CCTAAAAATC	1440
	CTGTCAGATC	CCTATGCTGA	CCACTTCCTT	TCAGATCAAC	AGCTGCCCTT	ACTCGAGCTC	1500
40	AAGCTTCGAA	TTCTGCAGTC	GACGGTACCG	CGGGCGCTAA	CTAATAGCCC	ATTCTCCAAG	1560
	GTACGTAGCG	GGGATCAATT	CCGCCCCCCC	CCTAACGTAA	CTGGCCGAAG	CCGCTTGGAA	1620
	TAAGGCCGGT	GTGCGTTGT	CTATATGTTA	TTTCCACCA	TATTGCCGTC	TTTGGCAAT	1680
45	GTGAGGGCCC	GGAAACCTGG	CCCTGTCTTC	TTGACGAGCA	TTCCTAGGGG	TCTTTCCCCT	1740
	CTCGCCAAAG	GAATGCAAGG	TCTGTTGAAT	GTGCGAAGG	AAGCAGTTCC	TCTGGAAGCT	1800
50	TCTTGAAGAC	AAACAACGTC	TGTAGCGACC	CTTTCAGGC	AGCGGAACCC	CCCACCTGGC	1860
	GACAGGTGCC	TCTGCGGCCA	AAAGCCACGT	GTATAAGATA	CACCTGCAA	GGCGGCACAA	1920
	CCCCAGTGCC	ACGTTGTGAG	TTGGATAGTT	GTGAAAGAG	TCAAATGGCT	CTCCTCAAGC	1980
55	GTATTCAACA	AGGGGCTGAA	GGATGCCAG	AAGGTACCCC	ATTGTATGGG	ATCTGATCTG	2040
	GGGCCTCGGT	GCACATGCTT	TACATGTGTT	TAGTCGAGGT	AAAAAAACG	TCTAGGCC	2100
	CCGAACCACG	GGGACGTGGT	TTTCCTTGA	AAAACACGAT	ACGGGATCCA	CCGGTCGCCA	2160
60	CCATGGTAA	AGGAGAAGAA	CTTTTACAG	GAGTTGTCCC	AATTCTTGT	GAATTAGATG	2220
	GTGATGTTAA	TGGGCACAAA	TTTCTGTCA	GTGGAGAGGG	TGAAGGTGAT	GCAACATACG	2280
65	AAAAACTTAC	CCTTAAATT	ATTTGCACTA	CTGGAAAAC	ACCTGTTCCA	TGGCCAACAC	2340
	TTGTCACTAC	TTTCACTTAT	GGTGTCAAT	GCTTTCAAG	ATACCCAGAT	CATATGAAAC	2400

	GGCATGACTT TTTCAAGAGT GCCATGCCCG AAGGTTATGT ACAGGAAAGA ACTATATTTT	2460
5	TCAAAGATGA CGGAACTAC AAGACACGTG CTGAAGTCAA GTTTGAAGGT GATAACCCTTG	2520
	TTAATAGAAC CGAGTTAAA GGTATTGATT TTAAAGAAGA TGGAACACATT CTTGGACACA	2580
	AATTGGAATA CAACTATAAC TCACACAATG TATACATCAT GGCAGACAAA CAAAAGAATG	2640
10	GAACCAAAGT TAACTTCAAA ATTAGACACA ACATTGAAGA TGGAAGCGTT CAACTAGCAG	2700
	ACCATTATCA ACAAAATACT CCAATTGGCG ATGGCCCTGT CCTTTTACCA GACAACCATT	2760
15	ACCTGTCCAC ACAATCTGCC CTTTCGAAAG ATCCCAACGA AAAGAGAGAC CACATGGTCC	2820
	TTCTTGAGTT TGTAACAGCT GCTGGGATTA CACATGGCAT GGATGAACTA TACAAGTCCG	2880
	GATCTAGATA ACTGTATCGA TGGATCCGAA GGCAGGGACAA GCAGTGCAGT GGTGGACAGA	2940
20	AAGCAAGTGA TCTAGGCCAG CAGCCTCCCT AAAGGGACTT CAGCCCACAA AGCCAAACTT	3000
	GTGGCTTTAA TACAAGCTCT GTAAATGGTA AAAAAAAAAGTCTACACG GACAGCAGGT	3060
25	ATGCTCTTGC CACTGTACAG AGCAATATAC AGACAAAGAG AACTGTTGAC ATCTGCAGAG	3120
	AAAGACCTAA GATGCTGTGG CTAAAAGAAA TCAGATGGCA AATCTAACCG CCCAGGCATC	3180
	CTAAAGAGCA ATGATCCTGA CAGTCTGAAG ACTATCAAGT TATAGACAAA TTAAGACTGG	3240
30	TAAAAAAAAC CCTGTATAAA ATAGTAAAAA CTGAAAAAAG AAAACTAGTC CTCTCATGAG	3300
	AAGACAGACC TGACATCTAC TGAAAAATAG ACTTTACTGG AAAAAATATG TGTATGAATA	3360
35	CCTTCTAGTT TTTGTGAACG TTCTCAAGAT GGATAAAAAGC TTTTCCTTGT AAAACGAGAC	3420
	TGATCAGATA GTCATCAAGA AGATTGTTAA AGAAAATTTT CCAAGGTTCG GAGTGCCAAA	3480
	AGCAATAGTG TCAGATAATG GTCCTGCCCT TGTGCCCCAG GTAAGTCAGG GTGTGGCCAA	3540
40	GTATTAGAG GTCAAATGAA AATTCCATTG TGTGTACAGA CCTCAGAGCT CAGGAAAGAT	3600
	AAAAAAGAAT AAATAAAACT CTAAACAGAC CTTGACAAAAA TTAATCCTAG AGACTGGCAC	3660
45	AGACTTACTT GGTACTCCCT CCCCTGCCCT TATTAGAAC TGAGAATACT CCCTCTTGAT	3720
	TCGGTTTTAC TCTTTTAAG ATCCTTATG GGGCTCCTAT GCCATCACTG TCTTAAATGA	3780
	TGTGTTAAA CCTATGTTGT TATAATAATG ATCTATATGT TAAGTTAAA GGCTTGCAGG	3840
50	TGGTGCAGAA AGAAGTCTGG TCACAACTGG CTACAGTGAA CAAGCTGGGT ACCCCCAAGGA	3900
	CATCTTACCA GTTCCAGCCA GAGATCTGAT CTACGATCCC CGGGTCGACC CGGGTCGACC	3960
	CTGTGGAATG TGTGTCAAGT AGGGTGTGGA AAGTCCCCAG GCTCCCCAGC AGGCAGAACT	4020
55	ATGCAAAGCA TGCATCTCAA TTAGTCAGCA ACCAGGTGTG GAAAGTCCCC AGGCTCCCCA	4080
	GCAGGGCAGAA GTATGCAAAG CATGCATCTC ATTAGTCAG CAACCATAGT CCCGCCCTA	4140
60	ACTCCGCCCA TCCCGCCCT AACTCCGCC AGTTCCGCC ATTCTCCGCC CCATGGCTGA	4200
	CTAATTTTTT TTATTTATGC AGAGGCCGAG GCCGCCTCGG CCTCTGAGCT ATTCCAGAAC	4260
	TAGTGAGGAG GCTTTTTGG AGGCCTAGGC TTTTGCAAAA AGCTTCACGC TGCCGCAAGC	4320
65	ACTCAGGGCG CAAGGGCTGC TAAAGGAAGC GGAACACGT A GAAAGCCAGT CCGCAGAAC	4380

	GGTGCTGACC CCGGATGAAT GTCAGCTACT GGGCTATCTG GACAAGGGAA AACGCAAGCG	4440
	CAAAGAGAAA GCAGGTAGCT TGCAGTGGGC TTACATGGCG ATAGCTAGAC TGGGGCGTTT	4500
5	TATGGACAGC AAGCGAACCG GAATTGCCAG CTGGGGCGCC CTCTGGTAAG GTTGGGAAGC	4560
	CCTGCAAAGT AAACCTGGATG GCTTTCTTGC CGCCAAGGAT CTGATGGCGC AGGGGATCAA	4620
10	GATCTGATCA AGAGACAGGA TGAGGATCGT TTCCGATGAT TGAACAAGAT GGATTGCACG	4680
	CAGGTTCTCC GGCGCGTTGG GTGGAGAGGC TATTGGCTA TGACTGGCA CAACAGACAA	4740
	TCGGCTGCTC TGATGCCGCC GTGTTCCGGC TGTCAGCGCA GGGGCGCCCG GTTCTTTTG	4800
15	TCAAGACCGA CCTGTCCGGT GCCCTGAATG AACTGCAGGA CGAGGCAGCG CGGCTATCGT	4860
	GGCTGGCAC GACGGGGCGTT CCTTGCCAG CTGTGCTCGA CGTTGTCACT GAAGCGGGAA	4920
20	GGGACTGGCT GCTATTGGGC GAACTGCCGG GGCAGGATCT CCTGTCATCT CACTTGCTC	4980
	CTGCCGAGAA AGTATCCATC ATGGCTGATG CAATGCCGCG GCTGCATAACG CTTGATCCGG	5040
	CTACCTGCCA ATTGACCCAC CAAGCGAAAC ATCGCATCGA GCGAGCACGT ACTCGGATGG	5100
25	AAGCCGGTCT TGTCGATCAG GATGATCTGG ACGAAGAGCA TCAGGGGCTC GCGCCAGCCG	5160
	AACTGTTCGC CAGGCTCAAG GCGCGCATGC CCGACGGCGA GGATCTCGTC GTGACCCATG	5220
30	GCGATGCCTG CTTGCCGAAT ATCATGGTGG AAAATGGCCG CTTTCTGGA TTCATCGACT	5280
	GTGGCCGGCT GGGTGTGGCG GACCGCTATC AGGACATAGC GTTGGCTACC CGTGATATTG	5340
	CTGAAGAGCT TGGCGCGAA TGGGCTGACC GCTTCCTCGT GCTTTACGGT ATCGCCGCTC	5400
35	CCGATTGCA GCGCATCGCC TTCTATCGCC TTCTTGACGA GTTCTTCTGA GCGGGACTCT	5460
	GGGGTTCGAA ATGACCGACC AAGCGACGCC CAACCTGCCA TCACGAGATT TCGATTCCAC	5520
40	CGCCGCCCTTC TATGAAAGGT TGGGCTTCGG AATCGTTTC CGGGACGGAA TTCGTAATCT	5580
	GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGGCG GATCAAGAGC	5640
	TACCAACTCT TTTTCCGAAG GTAAGTGGCT TCAGCAGAGC GCAGATAACCA AATACTGTCC	5700
45	TTCTAGTGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC	5760
	TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG	5820
50	GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT	5880
	CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG	5940
	AGCATTGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG	6000
55	GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTT	6060
	ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTGTGA TGCTCGTCAG	6120
	GGGGCGGGAG CCTATGGAAA AACGCCAGCA ACGCCGAGAT GCGCCGCCTC GAGTACACCT	6180
60	GCGTCATGCT GAGACCCCTCA AGCCTCACTA AAAGGGTCCC TGCCTAGTTC TGTTTACTAA	6240
	TCTGCCCTTAT TCTGTTTTTG TTCCCATGTT AAAGATAGAG TAAATGCAGT ATTCTCCACAA	6300
	TAGAGATATA GACTTCTGAA ATTCTAAGAT TAGAATTATT TACAAGAAGA AGTGGGGAA	6359

(2) INFORMATION FOR SEQ ID NO:16:

5                   (i) SEQUENCE CHARACTERISTICS:  
                     (A) LENGTH: 6891 base pairs  
                     (B) TYPE: nucleic acid  
                     (C) STRANDEDNESS: single  
                     (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

15	TGAAGAATAA AAAATTACTG GCCTCTTGTG AGAACATGAA CTTTCACCTC GGAGCCCACC CCCTCCCATC TGGAAAACAT ACTTGAGAAA AACATTTCT GGAACAACCA CAGAATGTTT CAACAGGCCA GATGTATTGC CAAACACAGG ATATGACTCT TTGGTTGAGT AAATTTGTGG	60 120 180
20	TTGTTAAACT TCCCCTATTG CCTCCCCATT CCCCTCCCA GTTGTGGTT TTTCCCTTA AAAGCTTGTG AAAAATTGAA GTCGTCGTCG AGACTCCTCT ACCCTGTGCA AAGGTGTATG	240 300
25	AGTTTCGACC CCAGAGCTCT GTGTGCTTTC TGTTGCTGCT TTATTCGAC CCCAGAGCTC TGGTCTGTGT GCTTCATGT CGCTGCTTTA TTAAATCTTA CCTTCTACAT TTTATGTATG	360 420
30	GTCCTAGTGT CTTCTGGGT ACGCGGCTGT CCCGGGACTT GAGTGTCTGA GTGAGGGTCT TCCCTCGAGG GTCTTCATT TGGTACATGG GCCGGGAATT CGAGAATCTT TCATTTGGTG	480 540
35	CATTGGCCGG GAATTGAAA ATCTTCATT TGGTGCATTG GCCGGGAAAC AGCGCGACCA CCCAGAGGTC CTAGACCCAC TTAGAGGTAA GATTCTTGT TCTGTTTGG TCTGATGTCT	600 660
40	GTGTTCTGAT GTCTGTGTT TGTTCTAAG TCTGGTGCAGA TCGCAGTTTC AGTTTGCGG ACGCTCAGTG AGACCGCGCT CCGAGAGGGA GTGCGGGGTG GATAAGGATA GACGTGTCCA	720 780
45	GGTGTCCACC GTCCGTTCGC CCTGGGAGAC GTCCCAGGAG GAACAGGGGA GGATCAGGGAA CGCCTGGTGG ACCCCTTGA AGGCCAAGAG ACCATTGGG GTTGGAGAT CGTGGGTTCG	840 900
50	ACCTCGTGTG AGTCCCACCT CGTGCCAGT TGCGAGATCG TGGGTTCGAG TCCCACCTCG TGTTTTGTTG CGAGATCGTG GGTCGAGTC CCACCTCGCG TCTGGTCACG GGATCGTGGG TTCGAGTCCC	960 1020
55	ACCTCGTGTGTT TTGTTGCGAG ATCGTGGGTT CGAGTCCCAC CTCGCGTCTG GTCACGGGAT CGTGGGTTCG AGTCCCACCT CGTGCAGAGG GTCTCAATTG GCCGGCCTTA GAGAGGCCAT	1080 1140
60	CTGATTCTTC TGGTTCTCT TTTGCTTA GTCTCGTGC CGCTCTTGTGTT GTGACTACTG TTGGTAATTT TGTTGTTA CGTTGTTTGTGAGTCGT CTATGTTGTC TGTTACTATC	1200 1260
65	TTGTTTTGT TTGTTGTTA CGGTTCTGT GTGTGTCCTG TGTGTCTCTT TGTGTTGAGA CTTGGACTGA TGACTGACGA CTGTTTTAA GTTATGCCTT CTAAAATAAG CCTAAAAATC	1320 1380
	CTGTCAGATC CCTATGCTGA CCACCTCCTT TCAGATCAAC AGCTGCCCTT ACTCGAGCTC	1440
	AAGCTTCGAA TTCTGCAGTC GACGGTACCG CGGGGATCAA TTCCGCCCCC CCCCTAACGT	1500
		1560

	TACTGGCCGA AGCCGCTTGG AATAAGGCCG GTGTGCGTTT GTCTATATGT TATTTTCCAC	1620
	CATATTGCCG TCTTTGGCA ATGTGAGGGC CCGGAAACCT GGCCCTGTCT TCTTGACGAG	1680
5	CATTCTAGG GGTCTTCCC CTCTGCCAA AGGAATGCAA GGTCTGTTGA ATGTCGTGAA	1740
	GGAAGCAGTT CCTCTGGAAG CTTCTGAAG ACAAACACG TCTGTAGCGA CCCTTGCAG	1800
10	GCAGCGGAAC CCCCCACCTG GCGACAGGTG CCTCTGCGGC CAAAAGCCAC GTGTATAAGA	1860
	TACACCTGCA AAGGCGGCAC AACCCAGTG CCACGTTGTG AGTTGGATAG TTGTGGAAAG	1920
	AGTCAAATGG CTCTCCTCAA GCGTATTCAA CAAGGGCTG AAGGATGCCG AGAAGGTACC	1980
15	CCATTGTATG GGATCTGATC TGGGGCCTCG GTGCACATGC TTTACATGTG TTTAGTCGAG	2040
	GTAAAAAAC GTCTAGGCCG CCCGAACAC GGGGACGTGG TTTTCCTTTG AAAAACACGA	2100
20	GCGGGATCAA TTCCGGCCCC CCCCTAACGT TACTGGCCGA AGCCGCTTGG AATAAGGCCG	2160
	GTGTGCGTTT GTCTATATGT TATTTCCAC CATATTGCCG TCTTTGGCA ATGTGAGGGC	2220
	CCGGAAACCT GCCCCTGTCT TCTTGACGAG CATTCTAGG GGTCTTCCC CTCTGCCAA	2280
25	AGGAATGCAA GGTCTGTTGA ATGTCGTGAA GGAAGCAGTT CCTCTGGAAG CTTCTGAAAG	2340
	ACAAACAAACG TCTGTAGCGA CCCTTGCAG GCAGCGGAAC CCCCCACCTG GCGACAGGTG	2400
30	CCTCTGCGGC CAAAAGCCAC GTGTATAAGA TACACCTGCA AAGGCGGCAC AACCCAGTG	2460
	CCACGTTGTG AGTTGGATAG TTGTGGAAAG AGTCAAATGG CTCTCCTCAA GCGTATTCAA	2520
	CAAGGGCTG AAGGATGCCG AGAAGGTACC CCATTGTATG GGATCTGATC TGGGGCCTCG	2580
35	GTGCACATGC TTTACATGTG TTTAGTCGAG GTTAAAAAAA CGTCTAGGCC CCCCCGAACCA	2640
	CGGGGACGTG GTTTCCCTT GAAAAACACG ATACGGGATC CACCGGTGCG CACCATGGGT	2700
40	AAAGGAGAAG AACTTTCAC AGGAGTTGTC CCAATTCTTG TTGAATTAGA TGGTGATGTT	2760
	AATGGGCACA AATTTCTGT CAGTGGAGAG GGTGAAGGTG ATGCAACATA CGGAAAACCTT	2820
	ACCCCTAAAT TTATTTGCAC TACTGGAAAA CTACCTGTT CATGGCCAAC ACTTGTCACT	2880
45	ACTTTCACTT ATGGTGTCA ATGCTTTCA AGATAACCCAG ATCATATGAA ACGGCATGAC	2940
	TTTTCAAGA GTGCCATGCC CGAAGGTTAT GTACAGGAAA GAACTATATT TTTCAAAGAT	3000
	GACGGGAACG ACAAGACACG TGCTGAAGTC AAGTTGAAG GTGATACCCCT TGTTAATAGA	3060
50	ATCGAGTTAA AAGGTATTGA TTTAAAGAA GATGGAAACA TTCTTGGACA CAAATTGGAA	3120
	TACAACATA ACTCACACAA TGTATACATC ATGGCAGACA AACAAAAGAA TGGAACCAAA	3180
55	GTAACTTCA AAATTAGACA CAACATTGAA GATGGAAGCG TTCAACTAGC AGACCATTAT	3240
	CAACAAAATA CTCCAATTGG CGATGGCCCT GTCTTTTAC CAGACAACCA TTACCTGTCC	3300
	ACACAATCTG CCCTTCGAA AGATCCAAC GAAAAGAGAG ACCACATGGT CCTTCTTGAG	3360
60	TTTGTAAACAG CTGCTGGGAT TACACATGGC ATGGATGAAC TATACAAGTC CGGATCTAGA	3420
	TAACGTATC GATGGATCCG AAGGCGGGGA CAGCAGTGCA GTGGTGGACA GAAAGCAAGT	3480
	GATCTAGGCC AGCAGCCTCC CTAAAGGGAC TTCAGCCCAC AAAGCCAAAC TTGTGGCTTT	3540
65	AATACAAGCT CTGTAAATGG TAAAAAAA AAAGTCTACA CGGACAGCAG GTATGCTTT	3600

	GCCACTGTAC AGAGCAATAT ACAGACAAAG AGAACTGTT ACATCTGCAG AGAAAGACCT	3660
5	AAGATGCTGT GGCTAAAAGA AATCAGATGG CAAATCTAAC CGCCCAGGCA TCCTAAAGAG	3720
	CAATGATCCT GACAGTCTGA AGACTATCAA GTTATAGACA AATTAAGACT GGTAAAAAAA	3780
	ACCCGTATA AAATAGTAAA AACTGAAAAA AGAAAACTAG TCCTCTCATG AGAAGACAGA	3840
10	CCTGACATCT ACTGAAAAAT AGACTTTACT GGAAAAAATA TGTGTATGAA TACCTTCTAG	3900
	TTTTTGAA CGTTCTCAAG ATGGATAAAA GCTTTCCCT GTAAAACGAG ACTGATCAGA	3960
15	TAGTCATCAA GAAGATTGTT AAAGAAAATT TTCCAAGGTT CGGAGTGCCA AAAGCAATAG	4020
	TGTCAGATAA TGTCCTGCC TTTGTTGCC AGGTAAGTCA GGGTGTGGCC AAGTATTTAG	4080
	AGGTCAAATG AAAATTCCAT TGTGTGTACA GACCTCAGAG CTCAGGAAAG ATAAAAAAGA	4140
20	ATAAATAAAA CTCTAACAG ACCTTGACAA AATTAATCCT AGAGACTGGC ACAGACTTAC	4200
	TTGGTACTCC TTCCCCITGC CCTATTTAGA ACTGAGAATA CTCCCTCTTG ATTGGTTTT	4260
25	ACTCTTTTA AGATCCTTTA TGGGGCTCCT ATGCCATCAC TGTCTTAAAT GATGTGTTTA	4320
	AACCTATGTT GTTATAATAA TGATCTATAT GTTAAGTTAA AAGGCTTGCA GGTGGTGCAG	4380
	AAAGAAAGTCT GGTACAAACT GGCTACAGTG AACAAAGCTGG GTACCCCAAG GACATCTTAC	4440
30	CAGTTCCAGC CAGAGATCTG ATCTACGATC CCCGGTCGA CCCGGTCGA CCCTGTGGAA	4500
	TGTGTGTCAG TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG	4560
35	CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC CCAGGCTCCC CAGCAGGCAG	4620
	AAAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC	4680
	CATCCCGCCCC CTAACTCCGC CCAGTTCCGC CCATTCTCCG CCCCATGGCT GACTAATT	4740
40	TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG	4800
	AGGCTTTTT GGAGGCCTAG GCTTTGCAA AAAGCTTCAC GCTGCCGCAA GCACTCAGGG	4860
	CGCAAGGGCT GCTAAAGGAA CGGGAACACG TAGAAAGCCA GTCCGCAGAA ACGGTGCTGA	4920
45	CCCCGGATGA ATGTCAGCTA CTGGGCTATC TGGACAAGGG AAAACGCAAG CGCAAAGAGA	4980
	AAGCAGGTAG CTTGCAGTGG GCTTACATGG CGATAGCTAG ACTGGGGCGGT TTATGGACA	5040
	GCAAGCGAAC CGGAATTGCC AGCTGGGCG CCCTCTGGTA AGGTTGGGAA GCCCTGCCAA	5100
50	GTAAACTGGAA TGGCTTTCTT GCGCCAAGG ATCTGATGCC GCAGGGGATC AAGATCTGAT	5160
	CAAGAGACAG GATGAGGATC GTTTCGCATG ATTGAACAAG ATGGATTGCA CGCAGGTTCT	5220
55	CCGGCCGCTT GGGTGGAGAG GCTATTCCGC TATGACTGGG CACAACAGAC AATCGGCTGC	5280
	TCTGATGCCG CCGTGTCTCG GCTGTCAGCG CAGGGCGCC CGGTTCTTT TGTCAAGACC	5340
	GACCTGTCCG GTGCCCTGAA TGAACATGCAG GACGGAGGCAG CGCGGCTATC GTGGCTGGCC	5400
60	ACGACGGGCG TTCCCTGCC AGCTGTGCTC GACGTTGTCA CTGAAGCGGG AAGGGACTGG	5460
	CTGCTATTGG GCGAAGTGCC GGGCAGGAT CTCCGTCTCAT CTCACCTTGC TCCTGCCGAG	5520
65	AAAGTATCCA TCATGGCTGA TGCAATGCCG CGGCTGCATA CGCTTGATCC GGCTACCTGC	5580

	CCATTGACC ACCAAGCGAA ACATCGCATC GAGCGAGCAC GTACTCGGAT GGAAGCCGGT	5640
	CTTGTGATC AGGATGATCT GGACGAAGAG CATCAGGGC TCGCGCCAGC CGAACTGTT	5700
5	GCCAGGCTCA AGGCGCGCAT GCCCGACGGC GAGGATCTCG TCGTGACCCA TGGCGATGCC	5760
	TGCTTGCCGA ATATCATGGT GGAAAATGGC CGCTTTCTG GATTCATCGA CTGTGGCCGG	5820
10	CTGGGTGTGG CGGACCGCTA TCAGGACATA GCGTTGGCTA CCCGTGATAT TGCTGAAGAG	5880
	CTTGGCGCG AATGGGCTGA CCGCTTCCTC GTGCTTTACG GTATGCCGC TCCCGATTG	5940
	CAGCGCATCG CCTTCTATCG CCTTCTTGAC GAGTCTTCT GAGCGGGACT CTGGGGTTCG	6000
15	AAATGACCGA CCAAGCGACG CCCAACCTGC CATCACGAGA TTTCGATTCC ACCGCCGCCT	6060
	TCTATGAAAG GTTGGGCTTC GGAATCGTT TCCGGGACGG AATTCTGTAAT CTGCTGCTTG	6120
20	CAAACAAAAA AACCAACCGCT ACCAGCGGTG GTTTGTTGC CGGATCAAGA GCTACCAACT	6180
	CTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAAC CAAATACTGT CCTTCTAGTG	6240
	TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG	6300
25	CTAACCTGT TACCACTGGC TGCTGCCAGT GGCAGATAAGT CGTGTCTTAC CGGGTTGGAC	6360
	TCAAGACGAT AGTTACCGGA TAAGGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA	6420
30	CAGCCCAGCT TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA	6480
	GAAAGCGCCA CGCTTCCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG CGGCAGGGTC	6540
	GGAACACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT	6600
35	GTCGGTTTC GCCACCTCTG ACTTGAGCGT CGATTTTGT GATGCTCGTC AGGGGGCGG	6660
	AGCCTATGGA AAAACGCCAG CAACGCCAG ATGCCGCC TCGACTACAC CTGCGTCATG	6720
40	CTGAGACCCCT CAAGCCTCAC TAAAAGGGTC CCTGCCTAGT TCTGTTACT AATCTGCCTT	6780
	ATTCTGTTTT TGTTCCCATG TTAAAGATAG AGTAAATGCA GTATTCTCCA CATAGAGATA	6840
	TAGACTTCTG AAATTCTAAG ATTAGAATTA TTTACAAGAA GAACTGGGA A	6891

## 45 (2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6321 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: DNA (genomic)

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

60	TGAAGAATAA AAAATTACTG GCCTCTTGTG AGAACATGAA CTTTCACCTC GGAGCCCACC	60
	CCCTCCCACAT TGAAAACAT ACTTGAGAAA AACATTTCT GGAACAACCA CAGAATGTTT	120
	CAACAGGCCA GATGTATTGC CAAACACAGG ATATGACTCT TTGGTTGAGT AAATTGTGG	180
65	TTGTTAAACT TCCCCTATTG CCTCCCCATT CCCCTCCCA GTTTGTGGTT TTTTCCTTTA	240

	AAAGCTTGTG AAAAATTGA GTCGTCGTCG AGACTCCTCT ACCCTGTGCA AAGGTGTATG	300
	AGTTTCGACC CCAGAGCTCT GTGTGCTTTC TGTTGCTGCT TTATTCGAC CCCAGAGCTC	360
5	TGGTCTGTGT GCTTTCATGT CGCTGCTTTA TTAAATCTTA CCTTCTACAT TTTATGTATG	420
	GTCTCAGTGT CTTCTGGGT ACGCAGGCTGT CCCGGGACTT GAGTGTCTGA GTGAGGGTCT	480
10	TCCCTCGAGG GTCTTCATT TGTTACATGG GCCGGGAATT CGAGAACATCTT TCATTTGGTG	540
	CATTGGCCGG GAATTGAAA ATCTTCATT TGTTGCATTG GCCGGGAAAC AGCGCGACCA	600
	CCCAGAGGTC CTAGACCCAC TTAGAGGTA GATTCTTGT TCTGTTTG TCTGATGTCT	660
15	GTGTTCTGAT GTCTGTGTTG TGTTCTAAG TCTGGTGCAGA TCGCAGTTTC AGTTTGCGG	720
	ACGCTCAGTG AGACCGCGCT CCGAGAGGGA GTGCCGGGTG GATAAGGATA GACGTGTCCA	780
20	GGTGTCCACC GTCCGTTCGC CCTGGGAGAC GTCCCAGGAG AAACAGGGGA GGATCAGGGA	840
	CGCCTGGTGG ACCCCTTGA AGGCCAAGAG ACCATTGGG GTTGCAGAGAT CGTGGGTTCG	900
	AGTCCCACCT CGTCCCCAGT TGCGAGATCG TGGGTTCGAG TCCCACCTCG TGTTTGTTG	960
25	CGAGATCGTG GGTTCGAGTC CCACCTCGCG TCTGGTCACG GGATCGTGGG TTCGAGTCCC	1020
	ACCTCGTGT TTGTTGCGAG ATCGTGGTT CGAGTCCCAC CTCGCGTCTG GTCACGGGAT	1080
30	CGTGGGTTCG AGTCCCACCT CGTGCAGAGG GTCTCAATTG GCCGGCCTTA GAGAGGCCAT	1140
	CTGATTCTTC TGTTTCTCT TTTGCTTAA GTCTCGTGTGTC CGCTCTTGTGTT GTGACTACTG	1200
	TTTTCTAAA AATGGGACAA TCTGTGTCCA CTCCCCTTTC TCTGACTCTG GTTCTGTGCG	1260
35	TTGGTAATTG TGTTGTTA CGTTGTTT TGTGAGTCGT CTATGTTGTC TGTTACTATC	1320
	TTGTTTTGTTA TTGTTGGTTA CGGTTCTGT GTGTGTCCTG TGTGTCCTT TGTGTTTCAGA	1380
40	CTTGGACTGA TGACTGACGA CTGTTTTAA GTTATGCCTT CTAAAATAAG CCTAAAAATC	1440
	CTGTCAGATC CCTATGCTGA CCACCTCCTT TCAGATCAAC AGCTGCCCTT ACTCGAGCTC	1500
	AAGCTTCGAA TTCTGCAGTC GACGGTACCG CGGGGATCAA TTCCGCCCTT CCCCTAACGT	1560
45	TACTGGCCGA AGCCGCTTGG AATAAGGCCG GTGTGCGTT GTCTATATGT TATTTCCAC	1620
	CATATTGCCG TCTTTGGCA ATGTGAGGGC CCGGAAACCT GGCCCTGTCT TCTTGACGAG	1680
50	CATTCTTAGG GGTCTTTCCC CTCTCGCCAA AGGAATGCAA GGTCTGTTGA ATGTCGTGAA	1740
	GGAAGCAGTT CCTCTGGAAG CTTCTGAAG ACAAAACAACG TCTGTAGCGA CCCTTGCAG	1800
	GCAGCGGAAC CCCCCACCTG GCGACAGGTG CCTCTCGCGC CAAAGCCAC GTGTATAAGA	1860
55	TACACCTGCA AAGGGCGGCAC AACCCCACTG CCACGTTGTG AGTTGGATAG TTGTGGAAAG	1920
	AGTCAAATGG CTCTCCTCAA GCGTATTCAA CAAGGGCTG AAGGATGCC AGAAGGTACC	1980
60	CCATTGTATG GGATCTGATC TGGGGCCTCG GTGCACATGC TTTACATGTG TTTAGTCGAG	2040
	GTAAAAAAA CGTCTAGGCC CCCCCGAACCA CGGGGACGTG GTTTCTTT GAAAAACACG	2100
	ATACGGGATC CACCGGTCGC CACCATGGGT AAAGGAGAAG AACTTTCAC AGGAGTTGTC	2160
65	CCAATTCTTG TTGAATTAGA TGGTGATGTT AATGGGCACA AATTTCTGT CAGTGGAGAG	2220

	GGTGAAGGTG ATGCAACATA CGGAAAAC TTACCTTAAAT TTACCTGCAC TACTGGAAAA	2280
	CTACCTGTT CATGGCCAAC ACTTGTCACT ACCTTCACCT ATGGTGTCA ATGCTTTCA	2340
5	AGATACCCAG ATCATATGAA ACGGCATGAC TTTTCAAGA GTGCCATGCC CGAAGGTTAT	2400
	GTACAGGAAA GAACTATATT TTTCAAAGAT GACGGGAACT ACAAGACACG TGCTGAAGTC	2460
10	AAGTTGAAG GTGATACCCCT TGTTAATAGA ATCGAGTTAA AAGGTATTGA TTTAAAGAA	2520
	GATGGAAACA TTCTGGACA CAAATTGGAA TACAACATA ACTCACACAA TGTATACATC	2580
	ATGGCAGACA AACAAAAGAA TGGAACCAAA GTTAACCTCA AAATTAGACA CAACATTGAA	2640
15	GATGGAAGCG TTCAACTAGC AGACCATTAT CAACAAAATA CTCCAATTGG CGATGGCCCT	2700
	GTCCTTTAC CAGACAAACCA TTACCTGTCC ACACAATCTG CCCTTCGAA AGATCCCAAC	2760
20	GAAAAGAGAG ACCACATGGT CCTTCTTGAG TTTGTAACAG CTGCTGGAT TACACATGGC	2820
	ATGGATGAAC TATACAAGTC CGGATCTAGA TAACTGTATC GATGGATCCG AAGGCGGGGA	2880
	CAGCAGTGCA GTGGTGGACA GAAAGCAAGT GATCTAGGCC AGCAGCCTCC CTAAAGGGAC	2940
25	TTCAGCCCAC AAAGCCAAAC TTGTGGCTTT AATACAAGCT CTGTAATGG TAAAAAAAAAA	3000
	AAAGTCTACA CGGACAGCAG GTATGCTTT GCCACTGTAC AGAGCAATAT ACAGACAAAG	3060
30	AGAACTGTT ACATCTGCAG AGAAAGACCT AAGATGCTGT GGCTAAAAGA AATCAGATGG	3120
	CAAATCTAAC CGCCCAGGCA TCCTAAAGAG CAATGATCCT GACAGTCTGA AGACTATCAA	3180
	GTTATAGACA AATTAAGACT GGTAAAAAAA ACCCTGTATA AAATAGTAAA AACTGAAAAAA	3240
35	AGAAAACTAG TCCTCTCATG AGAAGACAGA CCTGACATCT ACTGAAAAAT AGACTTTACT	3300
	GGAAAAAAATA TGTGTATGAA TACCTTCTAG TTTTGTGAA CGTTCTCAAG ATGGATAAAA	3360
40	GCTTTTCCTT GTAAAACGAG ACTGATCAGA TAGTCATCAA GAAGATTGTT AAAGAAAATT	3420
	TTCCAAGGTT CGGAGTGCCA AAAGCAATAG TGTAGATAA TGGTCCTGCC TTTGTTGCC	3480
	AGGTAAGTCA GGGTGTGGCC AAGTATTAG AGGTCAAATG AAAATTCCAT TGTGTGTACA	3540
	GACCTCAGAG CTCAGGAAAG ATAAGAAAAA CTCTAAACAG ACCTGACAA	3600
45	AATTAATCCT AGAGACTGGC ACAGACTTAC TTGGTACTCC TTCCCCCTTGC CCTATTAGA	3660
	ACTGAGAATA CTCCCTTTG ATTGGTTTT ACTCTTTTA AGATCCTTTA TGGGGCTCCT	3720
50	ATGCCATCAC TGTCTTAAAT GATGTGTAA AACCTATGTT GTTATAATAA TGATCTATAT	3780
	GTAAAGTTAA AAGGCTTGCA GGTGGTGCAG AAAGAAGTCT GGTCACAACT GGCTACAGTG	3840
	AACAAGCTGG GTACCCCAAG GACATCTTAC CAGTCCAGC CAGAGATCTG ATCTACGATC	3900
55	CCCGGGTCGA CCCGGTCGA CCCTGTGAA TGTGTGTAG TTAGGGTGTG GAAAGTCCCC	3960
	AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG	4020
60	TGGAAAGTCC CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC	4080
	AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC CCAGTTCCGC	4140
	CCATTCTCCG CCCCATGGCT GACTAATTT TTTTATTAT GCAGAGGCCG AGGCCGCCTC	4200
65	GGCCTCTGAG CTATTCCAGA AGTAGTGAGG AGGCTTTTT GGAGGCCTAG GCTTTGCAA	4260

	AAAGCTTCAC GCTGCCGCAA GCACTCAGGG CGCAAGGGCT GCTAAAGGAA GCGGAACACG	4320
5	TAGAAAGCCA GTCCGCAGAA ACGGTGCTGA CCCCCGGATGA ATGTCAGCTA CTGGGCTATC	4380
	TGGACAAGGG AAAACGCAAG CGCAAAGAGA AAGCAGGTAG CTTGCAGTGG GCTTACATGG	4440
	CGATAGCTAG ACTGGGGCGGT TTTATGGACA GCAAGCGAAC CGGAATTGCC AGCTGGGCG	4500
10	CCCTCTGGTA AGGTTGGAA GCCCTGCAAA GTAAACTGGA TGGCTTCCTT GCCGCCAAGG	4560
	ATCTGATGGC GCAGGGGATC AAGATCTGAT CAAGAGACAG GATGAGGATC GTTTCGCATG	4620
15	ATTGAACAAG ATGGATTGCA CGCAGGTTCT CCGGCCGCTT GGGTGGAGAG GCTATTCGGC	4680
	TATGACTGGG CACAACAGAC AATCGGCTGC TCTGATGCCG CCGTGTTCCG GCTGTCAGCG	4740
	CAGGGCGCC CGGTTCTTT TGTCAGGACC GACCTGTCCG GTGCCCTGAA TGAACATGCAG	4800
20	GACGAGGCAG CGCGGCTATC GTGGCTGGCC ACGACGGGCG TTCCCTGCGC AGCTGTGCTC	4860
	GACGTGTCA CTGAAGCGGG AAGGGACTGG CTGCTATTGG GCGAAGTGCC GGGGCAGGAT	4920
25	CTCCTGTCAT CTCACCTTGC TCCTGCCGAG AAAGTATCCA TCATGGCTGA TGCAATGCGG	4980
	CGGCTGCATA CGCTTGATCC GGCTACCTGC CCATTCGACC ACCAAGCGAA ACATCGCATC	5040
	GAGCGAGCAC GTACTCGGAT GGAAGCCGGT CTTGTCGATC AGGATGATCT GGACGAAGAG	5100
30	CATCAGGGGC TCGCGCCAGC CGAACTGTTG GCCAGGCTCA AGGCGCGCAT GCCCGACGGC	5160
	GAGGATCTCG TCGTGACCCA TGGCGATGCC TGCTGCCGA ATATCATGGT GGAAAATGGC	5220
35	CGCTTTCTG GATTGATCGA CTGTGGCCGG CTGGGTGTGG CGGACCGCTA TCAGGACATA	5280
	GCGTTGGCTA CCCGTGATAT TGCTGAAGAG CTTGGCGGCG AATGGGCTGA CCGCTTCCTC	5340
	GTGCTTACG GTATCGCCGC TCCCGATTG CAGCGCATCG CCTTCTATCG CCTTCTTGAC	5400
40	GAGTTCTTCT GAGCGGGACT CTGGGGTTCG AAATGACCGA CCAAGCGACG CCCAACCTGC	5460
	CATCACGAGA TTTCGATTCC ACCGCCGCCT TCTATGAAAG GTTGGGCTTC GGAATCGTT	5520
	TCCGGGACGG AATTGTAAT CTGCTGTTG CAAACAAAAA AACCAACCGCT ACCAGCGGTG	5580
45	GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTCCGA AGGTAACTGG CTTCAGCAGA	5640
	GCGCAGATAAC CAAATACTGT CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC	5700
50	TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAAGTGGC TGCTGCCAGT	5760
	GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCCAG	5820
	CGGTGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC GACCTACACC	5880
55	GAACTGAGAT ACCTACAGCG TGAGCATTGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG	5940
	GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA	6000
60	GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT	6060
	CGATTTTGT GATGCTCGTC AGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCCGAG	6120
	ATGCGCCGCC TCGAGTACAC CTGCGTCATG CTGAGACCCCT CAAGCCTCAC TAAAAGGGTC	6180
65	CCTGCCTAGT TCTGTTTACT AATCTGCCCT ATTCTGTTT TGTTCCCATG TTAAAGATAG	6240

AGTAAATGCA GTATTCTCCA CATAGAGATA TAGACTTCTG AAATTCTAAG ATTAGAATTA	6300
TTTACAAGAA GAAGTGGGGA A	6321

## 5 (2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 5754 base pairs  
 (B) TYPE: nucleic acid  
 10 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

20 TGAAGAATAA AAAATTACTG GCCTCTTGTG AGAACATGAA CTTTCACCTC GGAGCCCACC	60
CCCTCCCATC TGAAAACAT ACTTGAGAAA AACATTTCT GGAACAAACCA CAGAATGTTT	120
25 CAACAGGCCA GATGTATTGC CAAACACAGG ATATGACTCT TTGGTTGAGT AAATTGTGG	180
TTGTTAAACT TCCCCTATTC CCTCCCCATT CCCCTCCCA GTTGTGGTT TTTCTTTA	240
AAAGCTTGTG AAAAATTGAA GTCGTCGTCG AGACTCCTCT ACCCTGTGCA AAGGTGTATG	300
30 AGTTTCGACC CCAGAGCTCT GTGTGTTTC TGTTGCTGCT TTATTCGAC CCCAGAGCTC	360
TGGTCTGTGT GCTTCATGT CGCTGTTTA TAAATCTTA CCTTCTACAT TTTATGTATG	420
GTCTCAGTGT CTTCTGGGT ACGCGGCTGT CCCGGGACTT GAGTGTCTGA GTGAGGGTCT	480
35 TCCCCTCGAGG GTCTTCATT TGGTACATGG GCCGGGAATT CGAGAATCTT TCATTGGTG	540
CATTGGCCGG GAATTCGAAA ATCTTCATT TGGTGCATTG GCCGGGAAAC AGCGCGACCA	600
40 CCCAGAGGTC CTAGACCCAC TTAGAGGTAA GATTCTTGT TCTGTTTGG TCTGATGTCT	660
GTGTTCTGAT GTCTGTGTTC TGTTCTAAG TCTGGTGCAG TCGCAGTTTC AGTTTGCGG	720
45 ACGCTCAGTG AGACCGCGCT CCGAGAGGGA GTGCGGGGTG GATAAGGATA GACGTGTCCA	780
GGTGTCCACC GTCCGTTCGC CCTGGGAGAC GTCCCAGGAG GAACAGGGGA GGATCAGGGA	840
CGCCTGGTGG ACCCCTTGA AGGCCAAGAG ACCATTTGGG GTTGCAGAT CGTGGGTTCG	900
50 AGTCCCACCT CGTGCCCCAGT TGCGAGATCG TGGGTTCGAG TCCCACCTCG TGTTTGTG	960
CGAGATCGTG GGTCGAGTC CCACCTCGCG TCTGGTCACG GGATCGTGGG TTGAGTCGG	1020
55 ACCTCGTGT TTGTTGCGAG ATCGTGGGTT CGAGTCCCAC CTCGCGTCTG GTCACGGGAT	1080
CGTGGGTTCG AGTCCCACCT CGTGCAGAGG GTCTCAATTG GCCGGCTTA GAGAGGCCAT	1140
CTGATTCTTC TGGTTCTCT TTTGTCTTA GTCTCGTGTG CGCTCTTGT GTGACTACTG	1200
60 TTTTCTAAA AATGGGACAA TCTGTGTCCA CTCCCCTTTC TCTGACTCTG GTTCTGTGCG	1260
TTGGTAATTG TGGTTGTTA CGTTTGTGTT TGTGAGTCGT CTATGTTGTC TGTTACTATC	1320
65 TTGTTTTGT TTGTGGTTA CGGTTCTGT GTGTGTCTTG TGTGTCTTT TGTGTTCAAGA	1380
CTTGGACTGA TGACTGACGA CTGTTTTAA GTTATGCCTT CTAAAATAAG CCTAAAAATC	1440

	CTGTCAGATC CCTATGCTGA CCACCTCCTT TCAGATCAAC AGCTGCCCTT ACTCGAGCTC	1500
5	AAGCTTCGAA TTCTGCAGTC GACGGTACCG CGGGCCCGGG ATCCACCGGT CGCCACCATG	1560
	GGTAAAGGAG AAGAACTTTT CACAGGAGTT GTCCCAATT TCAGGAGTT AGATGGTGAT	1620
	GTAAATGGGC ACAAAATTTT TGTCAGTGGA GAGGGTGAAG GTGATGCAAC ATACGGAAAA	1680
10	CTTACCCCTTA AATTTATTTG CACTACTGGA AAACATACCTG TTCCATGGCC AACACTTGTC	1740
	ACTACTTTCA CTTATGGTGT TCAATGCTTT TCAAGATAACC CAGATCATAT GAAACGGCAT	1800
15	GACTTTTCA AGAGTGCCAT GCCCGAAGGT TATGTACAGG AAAGAACTAT ATTTTCAAA	1860
	GATGACGGGA ACTACAAGAC ACGTGCTGAA GTCAAGTTG AAGGTGATAC CCTTGTAAAT	1920
	AGAATCGAGT TAAAAGGTAT TGATTTAAA GAAGATGGAA ACATTCTTGG ACACAAATTG	1980
20	GAATACAAC TAAACTCACA CAATGTATAC ATCATGGCAG ACAAAACAAA GAATGGAACC	2040
	AAAGTTAACT TCAAAATTAG ACACAACATT GAAGATGGAA GCGTTCAACT AGCAGACCAT	2100
25	TATCAACAAA ATACTCCAAT TGGCGATGGC CCTGCTCTT TACCAAGACAA CCATTACCTG	2160
	TCCACACAAAT CTGCCCTTTC GAAAGATCCC AACGAAAAGA GAGACCACAT GGTCTTCTT	2220
	GAGTTTGTAA CAGCTGCTGG GATTACACAT GGCAATGGATG AACTATACAA GTCCGGATCT	2280
30	AGATAACTGT ATCGATGGAT CCGAAGGGCGG GGACAGCAGT GCAGTGGTGG ACAGAAAGCA	2340
	AGTGATCTAG GCCAGCAGCC TCCCTAAAGG GACTTCAGCC CACAAAGCCA AACTGTGGC	2400
35	TTTAATACAA GCTCTGTAAA TGGTAAAAAA AAAAAGTCT ACACGGACAG CAGGTATGCT	2460
	CTTGCCACTG TACAGAGCAA TATACTGACA AAGAGAACTG TTGACATCTG CAGAGAAAGA	2520
	CCTAAGATGC TGTGGCTAAA AGAAATCAGA TGGCAAATCT AACCGCCCAAG GCATCCTAAA	2580
40	GAGCAATGAT CCTGACAGTC TGAAGACTAT CAAGTTATAG ACAAAATTAAG ACTGGTAAAA	2640
	AAAACCTGT ATAAAATAGT AAAAAGTGA AAAAGAAAAC TAGTCCTCTC ATGAGAAAGAC	2700
	AGACCTGACA TCTACTGAAA AATAGACTTT ACTGGAAAAA ATATGTGTAT GAATACCTTC	2760
45	TAGTTTTGT GAACGTTCTC AAGATGGATA AAAGCTTTTC CTTGTAAAC GAGACTGATC	2820
	AGATAGTCAT CAAGAAGATT GTTAAAGAAA ATTTCCAAG GTTCGGAGTG CCAAAAGCAA	2880
50	TAGTGTCAAGA TAATGGTCCT GCCTTTGTTG CCCAGGTAAG TCAGGGTGTG GCCAAGTATT	2940
	TAGAGGTCAA ATGAAAATTC CATTGTGTGT ACAGACCTCA GAGCTCAGGA AAGATAAAAA	3000
	AGAATAAATA AAACCTCTAAA CAGACCTTGA CAAAATTAAT CCTAGAGACT GGCACAGACT	3060
55	TACTTGGTAC TCCTTCCCCCT TGCCCTATTT AGAACGTGAGA ATACTCCCTC TTGATTGGT	3120
	TTTACTCTTT TTAAGATCCT TTATGGGCT CCTATGCCAT CACTGTCTTA AATGATGTGT	3180
60	TTAACCTAT GTTGTATCAA TAATGATCTA TATGTTAAGT TAAAAGGCTT GCAGGTGGT	3240
	CAGAAAGAAG TCTGGTCACA ACTGGCTACA GTGAACAAAGC TGGGTACCCC AAGGACATCT	3300
	TACCAGTTCC AGCCAGAGAT CTGATCTACG ATCCCCGGGT CGACCCGGGT CGACCCTGTG	3360
65	GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA GAAGTATGCA	3420

	AAGCATGCAT CTCATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG	3480
	CAGAACTATG CAAAGCATGC ATCTCAATTA GTCAGCAACC ATAGTCCCGC CCCTAACTCC	3540
5	GCCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTC CCGCCCCATG GCTGACTAAT	3600
	TTTTTTTATT TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG	3660
10	AGGAGGCTTT TTTGGAGGCC TAGGCTTTG CAAAAAGCTT CACGCTGCCG CAAGCACTCA	3720
	GGGCGCAAGG GCTGCTAAAG GAAGCGGAAC ACGTAGAAAG CCAGTCCGCA GAAACGGTGC	3780
	TGACCCCGGA TGAAATGTCAG CTACTGGGCT ATCTGGACAA GGGAAAACGC AAGCGCAAAG	3840
15	AGAAAAGCAGG TAGCTTGCAG TGGGCTTACA TGGCGATAGC TAGACTGGGC GGTTTATGG	3900
	ACAGCAAGCG AACCGGAATT GCCAGCTGGG GC GCCCTCTG GTAAGGTTGG GAAGCCCTGC	3960
20	AAAGTAAACT GGATGGCTT CTTGCCGCCA AGGATCTGAT GGCGCAGGGG ATCAAGATCT	4020
	GATCAAGAGA CAGGATGAGG ATCGTTCGC ATGATTGAAC AAGATGGATT GCACGCAGGT	4080
	TCTCCGGCCG CTTGGGTGGA GAGGCTATTG GGCTATGACT GGGCACAAACA GACAATCGGC	4140
25	TGCTCTGATG CCGCCGTGTT CCGGCTGTCA GCGCAGGGGC GCCCGGTTCT TTTGTCAAG	4200
	ACCGACCTGT CCGGTGCCCT GAATGAACTG CAGGACGAGG CAGCGCGGCT ATCGTGGCTG	4260
30	GCCACGACGG GCGTTCCCTTG CGCAGCTGTG CTGACGTTG TCACTGAAGC GGGAAAGGGAC	4320
	TGGCTGCTAT TGGGCGAACT GCGGGGGCAG GATCTCCTGT CATCTCACCT TGCTCCTGCC	4380
	GAGAAAGTAT CCATCATGGC TGATGCAATG CGGCGGCTGC ATACGCTTGA TCCGGCTACC	4440
35	TGCCCATTCG ACCACCAAGC GAAACATCGC ATCGAGCGAG CACGTACTCG GATGGAAGCC	4500
	GGTCTTGTGCG ATCAGGATGA TCTGGACGAA GAGCATCAGG GGCTCGGCC AGCCGAAC TG	4560
40	TTCGCCAGGC TCAAGGCGCG CATGCCGAC GGCGAGGATC TCGTCGTGAC CCATGGCGAT	4620
	GCCTGCTTGC CGAATATCAT GGTGGAAAAT GGCGCTTTT CTGGATTCAT CGACTGTGGC	4680
	CGGCTGGGTG TGGCGGACCG CTATCAGGAC ATAGCCTTGG CTACCCGTGA TATTGCTGAA	4740
45	GAGCTTGGCG GCGAATGGGC TGACCGCTTC CTGGTCTT ACGGTATCGC CGCTCCCGAT	4800
	TCGCAGCGCA TCGCCTTCTA TCGCCTTCTT GACGAGTTCT TCTGAGCGGG ACTCTGGGT	4860
	TCGAAATGAC CGACCAAGCG ACGCCCAACC TGCCATCACG AGATTCGAT TCCACGCCG	4920
50	CCTTCTATGA AAGGTTGGGC TTCGGAATCG TTTCCGGGA CGGAATTCTG AATCTGCTGC	4980
	TTGCAAACAA AAAAACCAACC GCTACCAGCG GTGGTTTGTG TGCCGGATCA AGAGCTACCA	5040
55	ACTCTTTTC CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC TGTCTTCTA	5100
	GTGTAGCCGT AGTTAGGCCA CCACCTCAAG AACTCTGTAG CACCGCCTAC ATACCTCGCT	5160
	CTGCTAATCC TGTTACCAAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT TACCGGGTTG	5220
60	GACTCAAGAC GATAAGT TACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG GGGTTCGTGC	5280
	ACACAGCCCA GCTTGGAGCG AACGACCTAC ACCGAAC TGA GATA CCTACA GCGTGAGCAT	5340
	TGAGAAAGCG CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG	5400
65	GTCGGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT	5460

	CCTGTGGGT TTGCCACCT CTGACTTGAG CGTCGATTT TGTGATGCTC GTCAGGGGG	5520
5	CGGAGCCTAT GGAAAAACGC CAGCAACGCC GAGATGCGCC GCCTCGAGTA CACCTGCGTC	5580
	ATGCTGAGAC CCTCAAGCCT CACTAAAAGG GTCCCTGCCT AGTTCTGTT ACTAATCTGC	5640
	CTTATTCTGT TTTGTTCCC ATGTTAAAGA TAGAGTAAAT GCAGTATTCT CCACATAGAG	5700
10	ATATAGACTT CTGAAATTCT AAGATTAGAA TTATTTACAA GAAGAAGTGG GGAA	5754

## (2) INFORMATION FOR SEQ ID NO:19:

15 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 5754 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: DNA (genomic)

## 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

	TGAAGAATAA AAAATTACTG GCCTCTGTG AGAACATGAA CTTTCACCTC GGAGCCCCACC	60
30	CCCTCCCATC TGAAAACAT ACTTGAGAAA AACATTTCT GGAACAACCA CAGAATGTTT	120
	CAACAGGCCA GATGTATTGC CAAACACAGG ATATGACTCT TTGGTTGAGT AAATTTGTGG	180
	TTGTTAAACT TCCCCTATT CCTCCCCATT CCCCTCCCA GTTTGTGGTT TTTTCCTTTA	240
35	AAAGCTTGTG AAAAATTGA GTCGTCGTCG AGACTCCTCT ACCCTGTGCA AAGGTGTATG	300
	AGTTTCGACC CCAGAGCTCT GTGTGTTTC TTGTCGCTGCT TTATTCGAC CCCAGAGCTC	360
40	TGGTCTGTGT GCTTCATGT CGCTGCTTTA TTAAATCTTA CCTTCTACAT TTTATGTATG	420
	GTCTCAGTGT CTTCTGGGT ACGCGGCTGT CCCGGGACTT GAGTGTCTGA GTGAGGGTCT	480
	TCCCTCGAGG GTCTTCATT TGGTACATGG GCCGGGAATT CGAGAATCTT TCATTTGGTG	540
45	CATTGGCCGG GAATTCGAAA ATCTTCATT TGGTGCATTG GCCGGGAAAC AGCGCGACCA	600
	CCCAGAGGTC CTAGACCCAC TTAGAGGTAA GATTCTTGT TCTGTTTGG TCTGATGTCT	660
50	GTGTTCTGAT GTCTGTGTT TCCTTCTAAG TCTGGTGCAG TCGCAGTTTC AGTTTGCGG	720
	ACGCTCAGTG AGACCGCGCT CCGAGAGGGA GTGCGGGGTG GATAAGGATA GACGTGTCCA	780
	GGTGTCCACC GTCCGTTCGC CCTGGGAGAC GTCCCAGGAG GAACAGGGGA GGATCAGGGA	840
55	CGCCTGGTGG ACCCCTTGA AGGCCAAGAG ACCATTTGGG GTTGCAGAGAT CGTGGGTTCG	900
	AGTCCCACCT CGTGCCAGT TGCGAGATCG TGGGTTCGAG TCCCACCTCG TGTTTGTG	960
60	CGAGATCGTG GGTCGAGTC CCACCTCGCG TCTGGTCACG GGATCGTGGG TTCGAGTCCC	1020
	ACCTCGTGTG TTGTTGCGAG ATCGTGGGTT CGAGTCCCAC CTCGCGTCTG GTCACGGGAT	1080
	CGTGGGTTCG AGTCCCACCT CGTGCAGAGG GTCTCAATTG GCCGGCCTTA GAGAGGCCAT	1140
65	CTGATTCTTC TGGTTTCTCT TTTTGTCTTA GTCTCGTGTG CGCTCTGTGTT GTGACTACTG	1200

	TTTTTCTAAA AATGGGACAA TCTGTGTCCA CTCCCCTTTC TCTGACTCTG GTTCTGTCGC	1260
	TTGGTAATT TGTTTGTATA CGTTTGTGTT TGAGTCGT CTATGTTGTC TGTTACTATC	1320
5	TTGTTTTGT TTGTGGTTA CGGTTCTGT GTGTGTCTTG TGTGTCTCTT TGTGTCAGA	1380
	CTTGGACTGA TGACTGACGA CTGTTTTAA GTTATGCCTT CTAAAATAAG CCTAAAAATC	1440
10	CTGTCAGATC CCTATGCTGA CCACCTCCCT TCAGATCAAC AGCTGCCCTT ACTCGAGCTC	1500
	AAGCTTCGAA TTCTGCAGTC GACGGTACCG CGGGCCCGGG ATCCACCGGT CGCCACCAG	1560
	GGTAAAGGAG AAGAACCTTT CACTGGAGTT GTCCCAATT TGTTGAATT AGATGGTGAT	1620
15	GTTAATGGGC ACAAAATTTC TGTCAGTGGA GAGGGTGAAG GTGATGCAAC ATACGGAAA	1680
	CTTACCCCTTA AATTATTTG CACTACTGGA AAACCTACCTG TTCCATGGCC AACACTTGTC	1740
20	ACTACTTTCT CTTATGGTGT TCAATGCTT TCAAGATACC CAGATCATAT GAAACGGCAT	1800
	GACTTTTCA AGAGTGCAT GCCCGAAGGT TATGTACAGG AAAGAACTAT ATTTTCAAA	1860
	GATGACGGGA ACTACAAGAC ACGTGCTGAA GTCAAGTTG AAGGTGATAC CCTTGTAAAT	1920
25	AGAACATCGAGT AAAAGGTAT TGATTTAAA GAAGATGGAA ACATTCTGG ACACAAATTG	1980
	GAATACAAC TAAACTCACA CAATGTATAC ATCATGGCAG ACAACACAA GAATGGAACC	2040
30	AAAGTTAACT TCAAAATTAG ACACAAACATT GAAGATGGAA GCGTTCAACT AGCAGACCAT	2100
	TATCAACAAA ATACTCCAAT TGGCGATGGC CCTGTCCCTT TACCAGACAA CCATTACCTG	2160
	TCCACACAAT CTGCCCTTTC GAAAGATCCC AACGAAAAGA GAGACCACAT GGTCTTCTT	2220
35	GAGTTTGTAA CAGCTGCTGG GATTACACAT GGCGATGGATG AACTATACAA GTCCGGATCT	2280
	AGATAACTGT ATCGATGGAT CCGAAGGCAG GGACAGCAGT GCAGTGGTGG ACAGAAAGCA	2340
	AGTGTAGCTAG GCCAGCAGCC TCCCTAAAGG GACTTCAGCC CACAAAGCCA AACTTGTGGC	2400
40	TTTAATACAA GCTCTGTAAA TGGTAAAAAA AAAAAAGTCT ACACGGACAG CAGGTATGCT	2460
	CTTGCCACTG TACAGAGCAA TATACAGACA AAGAGAACTG TTGACATCTG CAGAGAAAAGA	2520
	CCTAAAGATGC TGTGGCTAAA AGAAATCAGA TGGCAAATCT AACCGCCAG GCATCCTAAA	2580
45	GAGCAATGAT CCTGACAGTC TGAAGACTAT CAAGTTATAG ACAAAATTAG ACTGGTAAA	2640
	AAAACCCCTGT ATAAAATAGT AAAAAGTGA AAAAGAAAAC TAGTCCTCTC ATGAGAAGAC	2700
50	AGACCTGACA TCTACTGAAA AATAGACTTT ACTGGAAAAA ATATGTGTAT GAATACCTTC	2760
	TAGTTTTGT GAACGTTCTC AAGATGGATA AAAGCTTTTC CTTGTAAAAC GAGACTGATC	2820
	AGATAGTCAT CAAGAAGATT GTTAAAGAAA ATTTTCCAAG GTTCGGAGTG CCAAAAGCAA	2880
55	TAGTGTCAAGA TAATGGTCCT GCCTTGTG CCCAGGTAAG TCAGGGTGTG GCCAAGTATT	2940
	TAGAGGTCAA ATGAAAATTC CATTGTGTGT ACAGACCTCA GAGCTCAGGA AAGATAAAAA	3000
60	AGAATAAATA AAACTCTAAA CAGACCTTGA CAAAATTAAT CCTAGAGACT GGCACAGACT	3060
	TACTTGGTAC TCCTTCCCCCT TGCCCTATTT AGAACTGAGA ATACTCCCTC TTGATTCGGT	3120
	TTTACTCTTT TTAAGATCCT TTATGGGCT CCTATGCCAT CACTGTCTTA AATGATGTGT	3180
65	TTAAACCTAT GTTGTATATAA TAATGATCTA TATGTTAAGT TAAAAGGCTT GCAGGTGGTG	3240

	CAGAAAGAAG TCTGGTCACA ACTGGCTACA GTGAACAAAGC TGGGTACCCC AAGGACATCT	3300
5	TACCAGTTCC AGCCAGAGAT CTGATCTACG ATCCCCGGGT CGACCCGGGT CGACCCCTGTG	3360
	GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA GAAGTATGCA	3420
	AAGCATGCAT CTCAATTAGT CAGCAACCAAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG	3480
10	CAGAAGTATG CAAAGCATGC ATCTCAATTA GTCAGCAACC ATAGTCCCCC CCCTAACTCC	3540
	GCCCCATCCCC CCCCTAACTC CGCCCAGTTC CGCCCATTC CCGCCCCATG GCTGACTAAT	3600
15	TTTTTTTATT TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG	3660
	AGGAGGCTTT TTTGGAGGCC TAGGCTTTG CAAAAAGCTT CACGCTGCCG CAAGCACTCA	3720
	GGGCGCAAGG GCTGCTAAAG GAAGCGGAAC ACGTAGAAAG CCAGTCCGCA GAAACGGTGC	3780
20	TGACCCCGGA TGAATGTCAG CTACTGGGCT ATCTGGACAA GGGAAAACGC AAGCGCAAAG	3840
	AGAAAGCAGG TAGCTTGCAAG TGGGCTTACA TGGCGATAGC TAGACTGGC GGTTTTATGG	3900
25	ACAGCAAGCG AACCGGAATT GCCAGCTGGG GCGCCCTCTG GTAAGGTTGG GAAGCCCTGC	3960
	AAAGTAAACT GGATGGCTTT CTTGCCGCCA AGGATCTGAT GGCAGGGGG ATCAAGATCT	4020
	GATCAAGAGA CAGGATGAGG ATCGTTTCGC ATGATTGAAC AAGATGGATT GCACGCAGGT	4080
30	TCTCCGGCCG CTTGGGTGGA GAGGCTATTG GGCTATGACT GGGCACAAACA GACAATCGC	4140
	TGCTCTGATG CCGCCGTGTT CCGGCTGTCA GCGCAGGGC GCGCCGGTTCT TTTTGTCAAG	4200
35	ACCGACCTGT CCGGTGCCCT GAATGAACTG CAGGACGAGG CAGCGCGGCT ATCGTGGCTG	4260
	GCCACGACGG GCGTTCCCTTG CGCAGCTGTG CTCGACGTTG TCACTGAAGC GGGAAAGGGAC	4320
	TGGCTGCTAT TGGGCGAAGT GCGGGGGCAG GATCTCCTGT CATCTCACCT TGCTCCTGCC	4380
40	GAGAAAGTAT CCATCATGGC TGATGCAATG CGGCGGCTGC ATACGCTTGA TCCGGCTACC	4440
	TGCCCATTCG ACCACCAAGC GAAACATCGC ATCGAGCGAG CACGTACTCG GATGGAAGCC	4500
	GGTCTTGTGCG ATCAGGATGA TCTGGACGAA GAGCATCAGG GGCTCGCGCC AGCCGAAC TG	4560
45	TTCGCCAGGC TCAAGGCGCG CATGCCGAC GGCGAGGATC TCGTCGTGAC CCATGGCGAT	4620
	GCCTGCTTGC CGAATATCAT GGTGGAAAAT GGCCGTTTT CTGGATTCAT CGACTGTGGC	4680
50	CGGCTGGGTG TGGCGGACCG CTATCAGGAC ATAGCGTTGG CTACCCGTGA TATTGCTGAA	4740
	GAGCTTGGCG GCGAATGGGC TGACCGCTTC CTCGTGCTT ACGGTATCGC CGCTCCCGAT	4800
	TCGCAGCGCA TCGCCTTCTA TCGCCTTCTT GACGAGTTCT TCTGAGCGGG ACTCTGGGT	4860
55	TCGAAATGAC CGACCAAGCG ACGCCCAACC TGCCATCACG AGATTCGAT TCCACGCCG	4920
	CCTTCTATGA AAGGTTGGC TTCGGAATCG TTTTCCGGGA CGGAATTGCGT AATCTGCTGC	4980
60	TTGCAAACAA AAAAACCAACC GCTACCAGCG GTGGTTGTT TGCCGGATCA AGAGCTACCA	5040
	ACTCTTTTC CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC TGTCTTCTA	5100
	GTGTAGCCGT AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC ATACCTCGCT	5160
65	CTGCTAATCC TGTTACCAAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT TACCGGGTTG	5220

	GACTCAAGAC GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG GGGTCGTGC	5280
	ACACAGCCC ACGTGGAGCG AACGACCTAC ACCGAACGTAA GATACCTACA GCGTGAGCAT	5340
5	TGAGAAAGCG CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCAGCAGG	5400
	GTCGGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCCTGGTA TCTTTATAGT	5460
10	CCTGTCGGGT TTCGCCACCT CTGACTTGAG CGTCGATTTC TGTGATGCTC GTCAGGGGG	5520
	CGGAGCCTAT GGAAAAAACGC CAGCAACGCC GAGATGCGCC GCCTCGAGTA CACCTGCGTC	5580
	ATGCTGAGAC CCTCAAGCCT CACTAAAAGG GTCCCTGCCT AGTTCTGTT ACTAATCTGC	5640
15	CTTATTCTGT TTTTGTTCCT ATGTTAAAGA TAGAGTAAAT GCAGTATTCT CCACATAGAG	5700
	ATATAGACTT CTGAAATTCT AAGATTAGAA TTATTTACAA GAAGAAGTGG GGAA	5754

20 (2) INFORMATION FOR SEQ ID NO:20:

- 20 (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 4958 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- 25 (ii) MOLECULE TYPE: DNA (genomic)

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

	AGGCAGGGAC AGCAGTGCAG TGGTGGACAG AAAGCAAGTG ATCTAGGCCA GCAGCCTCCC	60
35	TAAAGGGACT TCAGCCCCACA AAGCCAAACT TGTGGCTTTA ATACAAGCTC TGAAATGGT	120
	AAAAAAAAAAA AAGTCTACAC GGACAGCAGG TATGCTCTTG CCACTGTACA GAGCAATATA	180
40	CAGACAAAGA GAACTGTTGA CATCTGCAGA GAAAGACCTA AGATGCTGTG GCTAAAAGAA	240
	ATCAGATGGC AAATCTAACCC GCCCAGGCAT CCTAAAGAGC AATGATCCTG ACAGTCTGAA	300
45	GACTATCAAG TTATAGACAA ATTAAGACTG GTAAAAAAAAA CCCTGTATAA AATAGTAAAA	360
	ACTGAAAAAAA GAAAAGTAGT CCTCTCATGA GAAGACAGAC CTGACATCTA CTGAAAAATA	420
	GACTTTACTG GAAAAAATAT GTGTATGAAT ACCTTCTAGT TTTTGTGAAC GTTCTCAAGA	480
50	TGGATAAAAG CTTTCCTTG TAAAACGAGA CTGATCAGAT AGTCATCAAG AAGATTGTTA	540
	AAGAAAATTT TCCAAGGTTG GGAGTGCCAA AAGCAATAGT GTCAGATAAT GGTCTGCCT	600
55	TTGTTGCCA GGTAAAGTCAG GGTGTGCCA AGTATTTAGA GGTCAAATGA AAATTCCATT	660
	GTGTGTACAG ACCTCAGAGC TCAGGAAAGA TAAAAAAAGAA TAAATAAAAC TCTAAACAGA	720
	CCTTGACAAA ATTAATCCTA GAGACTGGCA CAGACTTACT TGGTACTCCT TCCCCCTGCC	780
60	CTATTAGAA CTGAGAATAC TCCCTCTTGA TTGGTTTTA CTCTTTTAA GATCCTTTAT	840
	GGGGCTCCTA TGCCATCACT GTCTTAAATG ATGTGTTAA ACCTATGTTG TTATAATAAT	900
65	GATCTATATG TTAAGTTAAA AGGCTTGCAG GTGGTGCAGA AAGAAGTCTG GTCACAACGT	960
	GCTACAGTGA ACAAGCTGGG TACCCCAAGG ACATCTTACC AGTTCCAGCC AGAGATCTGA	1020

	TCTACGATCC CCGGGTCGAC CCGGGTCGAC CCTGTGGAAT GTGTGTCAGT TAGGGTGTGG	1080
5	AAAGTCCCCA GGCTCCCCAG CAGGCAGAAG TATGCAAAGC ATGCATCTCA ATTAGTCAGC	1140
	AACCAGGTGT GGAAAGTCCC CAGGCTCCCC AGCAGGCAGA AGTATGCAA GCATGCATCT	1200
	CAATTAGTCA GCAACCATAG TCCC GCCCCT AACTCCGCC ATCCGCC CCC TAACTCCGCC	1260
10	CAGTTCCGCC CATTCTCCGC CCCATGGCTG ACTAATTTT TTTATTTATG CAGAGGCCGA	1320
	GGCCGCCCTCG GCCTCTGAGC TATTCCAGAA GTAGTGAGGA GGCTTTTTG GAGGCCTAGG	1380
15	CTTTTGCAA AAGCTTCACG CTGCCGCAAG CACTCAGGGC GCAAGGGCTG CTAAAGGAAG	1440
	CGGAACACGT AGAAAGCCAG TCCGCAGAAA CGGTGCTGAC CCCGGATGAA TGTCAGCTAC	1500
	TGGGCTATCT GGACAAGGGGA AAACGCAAGC GCAAAGAGAA AGCAGGTAGC TTGCAGTGGG	1560
20	CTTACATGGC GATAGCTAGA CTGGGCGGTT TTATGGACAG CAAGCGAAC GGAATTGCCA	1620
	GCTGGGGCGC CCTCTGGTAA GGTTGGGAAG CCCTGCAAAG TAAACTGGAT GGCTTTCTTG	1680
25	CCGCCAAGGA TCTGATGGCG CAGGGGATCA AGATCTGATC AAGAGACAGG ATGAGGATCG	1740
	TTTCGATGA TTGAACAAGA TGGATTGCAC GCAGGTTCTC CGGCCGCTTG GGTGGAGAGG	1800
	CTATTGGCT ATGACTGGGC ACAACAGACA ATCGGCTGCT CTGATGCCGC CGTGTCCGG	1860
30	CTGTCAGCGC AGGGGCGCCC GGTTCTTTT GTCAAGACCG ACCTGTCCGG TGCCCTGAAT	1920
	GAACATGCAGG ACGAGGCAGC GCGGCTATCG TGGCTGGCCA CGACGGGCGT TCCTGCGCA	1980
35	GCTGTGCTCG ACCTGGTCAC TGAAGCGGGA AGGGACTGGC TGCTATTGGG CGAAGTGCCG	2040
	GGGCAGGATC TCCTGTCATC TCACCTTGCT CCTGCCGAGA AAGTATCCAT CATGGCTGAT	2100
	GCAATGCGGC GGCTGCATAC GCTTGATCCG GCTACCTGCC CATTGACCA CCAAGCGAAA	2160
40	CATCGCATCG AGCGAGCACG TACTCGGATG GAAGCCGGTC TTGTCGATCA GGATGATCTG	2220
	GACGAAGAGC ATCAGGGGCT CGGCCAGCC GAACATGTTCG CCAGGCTCAA GGCGCGCATG	2280
	CCCGACGGCG AGGATCTCGT CGTGACCCAT GGCGATGCC GCTTGCCGAA TATCATGGTG	2340
45	GAAAATGGCC GCTTTCTGG ATTCAATCGAC TGTGGCCGGC TGGGTGTGGC GGACCGCTAT	2400
	CAGGACATAG CGTTGGCTAC CCGTGATATT GCTGAAGAGC TTGGCGGCGA ATGGGCTGAC	2460
50	CGCTTCTCG TGCTTACGG TATGCCGCT CCCGATTCCG AGCGCATCCG CTTCTATCGC	2520
	CTTCTTGACG AGTTCTTCTG AGCGGGACTC TGGGGTTCGA AATGACCGAC CAAGCGACGC	2580
	CCAACCTGCC ATCACGAGAT TTCAATTCCA CCGCCGCCCTT CTATGAAAGG TTGGGTTCG	2640
55	GAATCGTTT CCGGGACGGA ATTCAATCGAC TGCTGCTTGC AAACAAAAAA ACCACCGCTA	2700
	CCAGCGGTGG TTGTTTGCC GGATCAAGAG CTACCAACTC TTTTCCGAA GGTAACTGGC	2760
60	TTCAGCAGAG CGCAGATAACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC	2820
	TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT ACCAGTGGCT	2880
	GCTGCCAGTG GCGATAAGTC GTGTCTTACG GGGTTGGACT CAAGACGATA GTTACCGGAT	2940
65	AAGGGCGAGC GGTGGGGCTG AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG	3000

	ACCTACACCG AACTGAGATA CCTACAGCGT GAGCATTGAG AAAGCGCCAC GCTTCCCGAA	3060
	GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG	3120
5	GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG TCGGGTTTCG CCACCTCTGA	3180
	CTTGAGCGTC GATTTTGTG ATGCTCGTCA GGGGGCGGA GCCTATGGAA AAACGCCAGC	3240
10	AACGCCGAGA TGCGCCGCCT CGAGTACACC TGCGTCATGC TGAGACCCCTC AAGCCTCACT	3300
	AAAAGGGTCC CTGCCTAGTT CTGTTTACTA ATCTGCCTTA TTCTGTTTT GTTCCCATGT	3360
	TAAAGATAGA GTAAATGCAG TATTCTCCAC ATAGAGATAT AGACTTCTGA AATTCTAAGA	3420
15	TTAGAATTAT TTACAAGAAG AAGTGGGAA TGAAGAATAA AAAATTACTG GCCTCTTGTG	3480
	AGAACATGAA CTTTCACCTC GGAGCCCACC CCCTCCCATC TGGAAAACAT ACTTGAGAAA	3540
20	AACATTTCT GGAACAACCA CAGAATGTT CAACAGGCCA GATGTATTGC CAAACACAGG	3600
	ATATGACTCT TTGGTTGAGT AAATTTGTGG TTGTTAAACT TCCCCTATTG CCTCCCCATT	3660
	CCCCCTCCCA GTTTGTGGTT TTTCCCTTA AAAGCTTGTG AAAAATTGA GTCGTCGTG	3720
25	AGACTCCTCT ACCCTGTGCA AAGGTGTATG AGTTTCGACC CCAGAGCTCT GTGTGTTTC	3780
	TGTTGCTGCT TTATTCGAC CCCAGAGCTC TGGTCTGTGT GCTTCATGT CGCTGTTTA	3840
30	TTAAATCTTA CCTTCTACAT TTTATGTATG GTCTCACTGT CTTCTTGGGT ACGCAGCTGT	3900
	CCCGGGACTT GAGTGTCTGA GTGAGGGTCT TCCCTCGAGG GTCTTTCATT TGGTACATGG	3960
	GCCGGGAATT CGAGAATCTT TCATTTGGTG CATTGGCCGG GAATTCGAAA ATCTTCATT	4020
	TGGTGCATTG GCCGGGAAAC AGCGCGACCA CCCAGAGGTC CTAGACCCAC TTAGAGGTAA	4080
35	GATTCTTGT TCTGTTTGG TCTGATGTCT GTGTTCTGAT GTCTGTGTTG TGTTCTAAG	4140
	TCTGGTGCAGA TCGCAGTTTC AGTTTGCGG ACGCTCAGTG AGACCGCGCT CCGAGAGGGA	4200
40	GTGCGGGGTG GATAAGGATA GACGTGTCCA GGTGTCCACC GTCCGTTCCG CCTGGGAGAC	4260
	GTCCCAGGAG GAACAGGGGA GGATCAGGGA CGCCTGGTGG ACCCCTTGA AGGCCAAGAG	4320
45	ACCATTGGG GTTGCAGAT CGTGGGTTCG AGTCCCACCT CGTGCCAGT TGCGAGATCG	4380
	TGGGTTCGAG TCCCACCTCG TGTTTGTG CGAGATCGT GGTTCGAGTC CCACCTCGCG	4440
	TCTGGTCACG GGATCGTGGG TTCAAGTCCC ACCTCGTGTG TTGTTGCGAG ATCGTGGGTT	4500
50	CGAGTCCCAC CTCGCGTCTG GTCACGGGAT CGTGGGTTCG AGTCCCACCT CGTGCAGAGG	4560
	GTCTCAATTG GCCGGCCTTA GAGAGGCCAT CTGATTCTTC TGTTTCTCT TTTGTCTTA	4620
	GTCTCGTGTGTC CGCTCTTGTG GTGACTACTG TTTTCTAAA AATGGGACAA TCTGTGTCCA	4680
55	CTCCCCCTTC TCTGACTCTG GTTCTGTCGC TTGGTAATTG TGTTGTTTA CGTTGTTTT	4740
	TGTGAGTCGT CTATGTTGTC TGTTACTATC TTGTTTTGT TTGTTGTTTA CGGTTCTGT	4800
60	GTGTGTCTTG TGTGTCTCTT TGTGTTCAGA CTTGGACTGA TGACTGACGA CTGTTTTAA	4860
	GTTATGCCTT CTAAAAATAAG CCTAAAAATC CTGTCAGATC CCTATGCTGA CCACTCCCTT	4920
65	TCAGATCAAC AGCTGCCCTT ACGTATCGAT GGATCCGA	4958

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7080 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

15	GAATACAAGC TTGCATGCCT GCAGGTCGAC TCTAGAGGAT CTTGAAGAAT AAAAAATTAC TGGCCTCTTG TGAGAACATG AACCTTCACC TCGGAGCCA CCCCCCTCCCA TCTGGAAAAC	60 120
20	ATACTTGAGA AAAACATTT CTGGAACAAC CACAGAATGT TTCAACAGGC CAGATGTATT GCCAAACACA GGATATGACT CTTGGTTGA GTAAATTGT GGTTGTTAAA CTTCCCTAT	180 240
25	TCCCTCCCCA TTCCCCCTCC CAGTTGTGG TTTTTCCCTT TAAAAGCTTG TGAAAAATT GAGTCGTCGT CGAGACTCCT CTACCCGTG CAAAGGTGTA TGAGTTCGA CCCCAGAGCT	300 360
30	CTGTGTGCTT TCTGTTGCTG CTTTATTTG ACCCCCAGAGC TCTGGTCTGT GTGCTTCAT GTCGCTGCTT TATTAAATCT TACCTTCTAC ATTTTATGTA TGGTCTCAGT GTCTTCTTG	420 480
35	GTACGCGGCT GTCCCGGGAC TTGAGTGTCT GAGTGAGGGT CTTCCCTCGA GGGTCTTC TTTGGTACAT GGGCCGGGAA TTCGAGAATC TTTCATTGG TGCAATTGGCC GGGAAATTCGA	540 600
40	AAATCTTCAT TTTGGTGCAT TGGCCGGGAA ACAGCGCGAC CACCCAGAGG TCCTAGACCC ACTTAGAGGT AAGATTCTTT GTTCTGTTTT GGTCTGATGT CTGTGTTCTG ATGTCTGTGT	660 720
45	TCTGTTCTA AGTCTGGTGC GATCGCAGTT TCAGTTTG TGACGCTCAG TGAGACCGCG CTCCGAGAGG GAGTGGGGGG TGGATAAGGA TAGACGTGTC CAGGTGTCCA CCGTCCGTT GCCCTGGGAG ACGTCCCAGG AGGAACAGGG GAGGATCAGG GACCCCTGGT GGACCCCTTT	780 840 900
50	GAAGGCCAAG AGACCATTG GGGTTGCGAG ATCGTGGTT CGAGTCCCAC CATCGATGGT GCAGAGGGTC TCAATTGGCC GGCCTTAGAA TTACGGATCT AGCATGATTG ACAAAGATGG ATTGCACGCA GGTTCTCCGG CCGCTTGGGT GGAGAGGCTA TTGGCTATG ACTGGGCACA	960 1020 1080
55	ACAGACAATC GGCTGCTCTG ATGCCGCCGT GTTCCGGCTG TCAGCGCAGG GGCGCCCGGT TCTTTTGTC AAGACCGACC TGTCCGGTGC CCTGAATGAA CTGCAGGACG AGGCAGCGCG	1140 1200
60	GCTATCGTGG CTGGCCACGA CGGGCGTTCC TTGCGCAGCT GTGCTCGACG TTGTCACTGA AGCGGGAAAGG GACTGGCTGC TATTGGGCGA AGTGCCGGGG CAGGATCTCC TGTCATCTCA CCTTGCTCCT GCCGAGAAAG TATCCATCAT GGCTGATGCA ATGCGGCGGC TGCATACGCT	1260 1320 1380
65	TGATCCGGCT ACCTGCCCAT TCGACCACCA AGCGAAACAT CGCATCGAGC GAGCACGTAC TCGGATGGAA GCCGGTCTTG TCGATCAGGA TGATCTGGAC GAAGAGCATC AGGGGCTCGC GCCAGCCGAA CTGTTCGCCA GGCTCAAGGC GCGCATGCC GACGGCGAGG ATCTCGTCTG	1440 1500 1560

	GACCCATGGC GATGCCTGCT TGCCGAATAT CATGGTGGAA AATGGCCGCT TTTCTGGATT	1620
	CATCGACTGT GGCCGGCTGG GTGTGGCGGA CCGCTATCAG GACATAGCGT TGGCTACCCG	1680
5	TGATATTGCT GAAGAGCTTG GCGCGAATG GGCTGACCGC TTCTCGTGC TTTACGGTAT	1740
	CGCCGCTCCC GATTGCGAGC GCATGCCCTT CTATGCCCTT CTTGACGAGT TCTTCTGAGC	1800
10	GGGACTCTGG GGTCGTAAT GACCGACCAA GCGACGCCA ACCTGCCATC ACGAGATTTC	1860
	GATTCCACCG CCGCCTTCTA TGAAAGGTTG GGCTTCGGAG TTAGCTTGT TCTTTACTGT	1920
	TTGTCAATTTC TATTATTTCATA ATACAGAACAA ATAGCTCTA TAACTGAAAT ATATTGCTA	1980
15	TTGTATATTA TGATTGTCCC TCGAACCATG AACACTCCTC CAGCTGAATT TCACAATTCC	2040
	TCTGTCAATTCT GCCAGGCCAT TAAGTTATTTC ATGGAAGATC TTTGAGGAAC ACTGCAAGTT	2100
20	CATATCATAA ACACATTGAA AATTGAGTAT TGTTTGCAT TGTATGGAGC TATGTTTTGC	2160
	TGTATCCTCA GAAAAAAAAGT TTGTTATAAA GCATTCACAC CCATAAAAAG ATAGATTAA	2220
	ATATTCCAGC TATAGGAAAG AAAGTGCCTC TGCTCTTCAC TCTAGTCTCA GTTGGCTCCT	2280
25	TCACATGCAT GCTTCTTTAT TTCTCCTATT TTGTCAAGAA AATAATAGGT CACGTCTTGT	2340
	TCTCACTTAT GTCCCTGCCTA GCATGGCTCA GATGCACGTT GTAGATACAA GAAGGATCAA	2400
30	ATGAAACAGA CTTCTGGTCT GTTACTACAA CCATAGTAAT AAGCACACTA ACTAATAATT	2460
	GCTAATTATG TTTTCCATCT CTAAGGTTC CACATTTTC TGTTTCTTA AAGATCCCAT	2520
	TATCTGGTTG TAACTGAAGC TCAATGGAAC ATGAGCAATA TTTCCAGTC TTCTCTCCCA	2580
35	TCCAACAGTC CTGATGGATT AGCAGAACAG GCAGAAAACA CATTGTTACC CAGAATTAA	2640
	AACTAATATT TGCTCTCCAT TCAATCCAAA ATGGACCTAT TGAAACTAAA ATCTAACCCA	2700
	ATCCCATTAA ATGATTTCATA TGGCGTCAAA GGTCAAACCTT CTGAAGGGAA CCTGTGGGTG	2760
40	GGTCACAATT CAGGCTATAT ATTCCCCAGG GCTCAGCCAG TGTCTGTACA TACACAACGG	2820
	ATCCTGTGGA CAGCTCACCT AGCTGCAATG GCTACAGGCT CCCGGACGTC CCTGCTCCTG	2880
	GCTTTGGCC TGCTCTGCCT GCCCTGGCTT CAAGAGGGCA GTGCCTTCCC AACCATTCCC	2940
45	TTATCCAGGC TTTTGACAA CGCTATGCTC CGCGCCCATC GTCTGCACCA GCTGGCCTTT	3000
	GACACCTACC AGGAGTTGA AGAAGCCTAT ATCCCAAAGG AACAGAAAGTA TTCATTCTG	3060
50	CAGAACCCCCC AGACCTCCCT CTGTTCTCA GAGTCTATTG CGACACCCTC CAACAGGGAG	3120
	GAAACACAAAC AGAAATCCAA CCTAGAGCTG CTCCGCATCT CCCTGCTGCT CATCCAGTCG	3180
	TGGCTGGAGC CCGTGCAGTT CCTCAGGAGT GTCTTCGCCA ACAGCCTGGT GTACGGCGCC	3240
55	TCTGACAGCA ACGTCTATGA CCTCTAAAG GACCTAGAGG AAGGCATCCA AACGCTGATG	3300
	GGGAGGCTGG AAGATGGCAG CCCCCGGACT GGGCAGATCT TCAAGCAGAC CTACAGCAAG	3360
60	TTCGACACAA ACTCACACAA CGATGACCGCA CTACTCAAGA ACTACGGGCT GCTCTACTGC	3420
	TTCAGGAAGG ACATGGACAA GGTCGAGACA TTCCCTGCGCA TCGTGCAGTG CCGCTCTGTG	3480
	GAGGGCAGCT GTGGCTTCTA GCTGCCCGGG TGGCATCCTG TGACCCCTCC CCAGTGCCTC	3540
65	TCCTGGCCCT GGAAGTTGCC ACTCCAGTGC CCACCAGCCT TGTCCATAATA AAATTAAGTT	3600

	GCATCAAAAA AAAAAAAAAG CTAGCGGCCG CTAGACTTCT GAAATTCTAA GATTAGAATT	3660
5	ATTTACAAGA AGAAGTGGGG AATGAAGAAC AAAAAATTAC TGGCCTCTTG TGAGAACATG	3720
	AACTTTCACC TCGGAGCCCA CCCCCCTCCA TCTGGAAAAC ATACTTGAGA AAAACATTT	3780
	CTGGAACAAAC CACAGAACATGT TTCAACAGGC CAGATGTATT GCCAAACACA GGATATGACT	3840
10	CTTTGGTTGA GTAAATTGT GGTTGTTAAA CTTCCCCTAT TCCCTCCCCA TTCCCCCTCC	3900
	CAGTTTGTGG TTTTTCCCTT TAAAAGCTTG TGAAAAATTG GAGTCGTCGT CGAGACTCCT	3960
15	CTACCCCTGTG CAAAGGTGTA TGAGTTTCGA CCCCAGAGCT CTGTGTGCTT TCTGTTGCTG	4020
	CTTTATTCG ACCCCAGAGC TCTGGTCTGT GTGCTTCAT GTCGCTGCTT TATTAAATCT	4080
	TACCTTCTAC ATTTTATGTA TGGTCTCAGT GTCTTCTTGG GTACGCGGCT GTCCCGGGAC	4140
20	TTGAGTGTCT GAGTGAGGGT CTTCCCTCGA GGGCTTTCA TTTGGTACAT GGGCCGGGAA	4200
	TTCGAGAACATC TTTCATTGGA TGCATTGCC GGGATTGGA AAATCTTCA GATCCCCGGG	4260
25	TACCGAGCTC GAATTCCGGT CTCCCTATAG TGAGTCGTAT TAATTCGAT AAGCCAGCTG	4320
	CATTAATGAA TCGGCCAACG CGCGGGGAGA GGCGGTTGCA GTATTGGGG CTCTCCGCT	4380
	TCCTCGCTCA CTGACTCGCT GCGCTCGGTGCTG GGCAGCGGT ATCAGCTCAC	4440
30	TCAAAGCGG TAATACGGTT ATCCACAGAA TCAGGGATA ACGCAGGAAA GAACATGTGA	4500
	GCAAAAGGCC AGCAAAAGGC CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTCCAT	4560
	AGGCTCCGCC CCCCTGACGA GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAC	4620
35	CCGACAGGAC TATAAAGATA CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT GCGCTCTCCT	4680
	GTTCCGACCC TGCCGCTTAC CGGATACCTG TCCGCCCTTC TCCCTTCGGG AAGCGTGGCG	4740
40	CTTTCTCATA GCTCACGCTG TAGGTATCTC AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG	4800
	GGCTGTGTGC ACGAACCCCC CGTTCAAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT	4860
	CTTGAGTCCA ACCCGGTAAAG ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG	4920
45	ATTAGCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG GCCTAACTAC	4980
	GGCTACACTA GAAGGACAGT ATTTGGTATC TGCGCTCTGC TGAAGCCAGT TACCTTCGGA	5040
	AAAAGAGTTG GTAGCTCTTG ATCCGGAAA CAAACCACCG CTGGTAGCGG TGGTTTTTT	5100
50	GTTCGCAAGC AGCAGATTAC GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT	5160
	TCTACGGGGT CTGACGCTCA GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA	5220
55	TTATCAAAAA GGATCTTCAC CTAGATCCTT TAAATTAAA AATGAAGTTT TAAATCAATC	5280
	TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT GCTTAATCAG TGAGGCACCT	5340
60	ATCTCAGCGA TCTGTCTATT TCGTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA	5400
	ACTACGATAC GGGAGGGCTT ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA	5460
	CGCTCACCGG CTCCAGATTG ATCAGCAATA AACCAAGCCAG CGGAAAGGGC CGAGCGCAGA	5520
65	AGTGGTCTG CAACTTTATC CGCCTCCATC CAGTCTATTA ATTGTTGCCG GGAAGCTAGA	5580

	GTAAGTAGTT CGCCAGTTAA TAGTTGCGC AACGTTGTTG CCATTGCTAC AGGCATCGTG	5640
	GTGTCACGCT CGTCGTTGG TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCAGA	5700
5	GTTACATGAT CCCCATGTT GTGAAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT	5760
	GTCAGAAAGTA AGTTGGCCGC AGTGTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT	5820
10	CTTACTGTCA TGCCATCCGT AAGATGCTTT TCTGTGACTG GTGAGTACTC AACCAAGTCA	5880
	TTCTGAGAAT AGTGTATGCG GCGACCGAGT TGCTCTGCC CGGCGTCAAT ACGGGATAAT	5940
	ACCGCGCCAC ATAGCAGAAC TTTAAAAGTG CTCATCATTG GAAAACGTTT TTGGGGCGA	6000
15	AAACTCTCAA GGATCTTACC GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC	6060
	AACTGATCTT CAGCATCTTT TACITTCACC AGCGTTCTG GGTGAGCAAA AACAGGAAGG	6120
20	CAAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC	6180
	CTTTTCAAT ATTATTGAAG CATTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT	6240
	GAATGTATTT AGAAAAATAA ACAAAATAGGG GTTCCCGCGA CATTTCGGGG AAAAGTGCCA	6300
25	CCTGACGTCT AAGAAACCAT TATTATCATG ACATTAACCT ATAAAAATAG GCGTATCACG	6360
	AGGCCCTTTC GTCTCGCGCG TTTCGGTGAT GACGGTAAA ACCTCTGACA CATGCAGCTC	6420
30	CCGGAGACGG TCACAGCTTG TCTGTAAGCG GATGCCGGGA GCAGACAAGC CCGTCAGGGC	6480
	GCGTCAGCGG GTGTTGGCGG GTGTCGGGCG TGGCTTAACT ATGCGGCATC AGAGCAGATT	6540
	GTACTGAGAG TGCACCATAT CGACGCTCTC CCTTATGCGA CTCTGCATT AGGAAGCAGC	6600
35	CCAGTAGTAG GTTGAGGCCG TTGAGCACCG CCGCCGCAAG GAATGGTGCA AGGAGATGGC	6660
	GCCCAACAGT CCCCCGGCCA CGGGGCGCTGC CACCATACCC ACGCCGAAAC AAGCGCTCAT	6720
	GAGCCCCGAAG TGGCGAGCCC GATCTTCCCC ATCGGTGATG TCGGCATAT AGGCGCCAGC	6780
40	AACCGCACCT GTGGCGCCGG TGATGCCGG CACGATGCGT CCGGCAGTAGA GGATCTGGCT	6840
	AGCGATGACC CTGCTGATTG GTTCGCTGAC CATTCCGGG GTGCGGAACG GCGTTACCAG	6900
	AAACTCAGAA GGTTCGTCCA ACCAAACCGA CTCTGACGGC AGTTTACGAG AGAGATGATA	6960
45	GGGTCTGCTT CAGTAAGCCA GATGCTACAC AATTAGGCTT GTACATATTG TCGTTAGAAC	7020
	GCGGCTACAA TTAATACATA ACCTTATGTA TCATACACAT ACGATTTAGG TGACACTATA	7080
50	(2) INFORMATION FOR SEQ ID NO:22:	

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 6795 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AATGAAAGAC CCCACCTGTA GGTTGGCAA GCTAGCTTAA GTAACGCCAT TTTGCAAGGC

60

	ATGGAAAAAT ACATAACTGA GAATAGAGAA GTTCAGATCA AGGTCAAGGAA CAGATGGAAC	120
5	AGCTGAATAT GGGCCAAACA GGATATCTGT GGTAAGCAGT TCCTGCCCG GCTCAGGGCC	180
	AAGAACAGAT GGAACAGCTG AATATGGGCC AAACAGGATA TCTGTGGTAA GCAGTTCTG	240
	CCCCGGCTCA GGGCCAAGAA CAGATGGTCC CCAGATGCGG TCCAGCCCTC AGCAGTTCT	300
10	AGAGAACCAT CAGATGTTTC CAGGGTCCCC CAAGGACCTG AAATGACCCCT GTGCCTTATT	360
	TGAACTAACCC AATCAGTTCG CTTCTCGCTT CTGTTCGCGC GCTTCTGCTC CCCGAGCTCA	420
15	ATAAAAGAGC CCACAAACCCC TCACTCGGGG CGCCAGTCCT CCGATTGACT GAGTCGCCCG	480
	GGTACCCGTG TATCCAATAA ACCCTCTTGC AGTTGCATCC GACTTGTGGT CTCGCTGTT	540
	CTTGGGAGGG TCTCCTCTGA GTGATTGACT ACCCGTCAGC GGGGGTCTTT CATTGGGGG	600
20	CTCGTCCGGG ATCGGGAGAC CCCTGCCAG GGACCACCGA CCCACCACCG GGAGGTAAGC	660
	TGGCCAGCAA CTTATCTGTG TCTGTCGGAT TGTCTAGTGT CTATGACTGA TTTTATGCGC	720
25	CTGCGTCGGT ACTAGTTAGC TAACTAGCTC TGTATCTGGC GGACCCGTGG TGGAACTGAC	780
	GAGTTCGGAA CACCCGGCCG CAACCTGGG AGACGTCCCA GGAGGAACAG GGGAGGATCA	840
	GGGACGCCCTG GTGGACCCCT TTGAAGGCCA AGAGACCATT TGGGGTTGCG AGATCGTGGG	900
30	TTCGAGTCCC ACCTCGTGCC CAGTTGCGAG ATCGTGGGTT CGAGTCCCAC CTCGTGTTT	960
	GTTGCGAGAT CGTGGGTTCG AGTCCCACCT CGCGTCTGGT CACGGGATCG TGGGTTCGAG	1020
	TCCCACCTCG TGTTTGTG CGAGATCGTG GGTCGAGTC CCACCTCGCG TCTGGTCACG	1080
35	GGATCGTGGG TTCGAGTCCC ACCTCGTGCA GAGGGTCTCA ATTGGCCGGC CTTAGAGAGG	1140
	CCATCTGATT CTTCTGGTTT CTCTTTTGT CTTAGTCTCG TGTCCGCTCT TGTGTGACT	1200
40	ACTGTTTTTC TAAAAATGGG ACAATCTGTG TCCACTCCCC TTTCTCTGAC TCTGGTTCTG	1260
	TCGCTTGGTA ATTTGTTTG TTACGTTTG TTTTGTGAG TCGTCTATGT TGTCTGTTAC	1320
	TATCTTGTGTT TTGTTGTGG TTTACGGTTT CTGTTGTGTT CTTGTGTGTC TCTTTGTGTT	1380
45	CAGACTTGGG CTGATGACTG ACGACTGTTT TTAAGTTATG CCTTCTAAAA TAAGCCTAAA	1440
	AATCCTGTCA GATCCCTATG CTGACCACTT CCTTCAGAT CAACAGCTGC CCTTACTCGA	1500
50	GCTCAAGCTT CGAATTCTGC AGTCGACGGT ACCGGGGCCG CTAACTAATA GCCCATTCTC	1560
	CAAGGTACGT AGCGGGGATC AATTCCGCC CCCCCCTAAC GTTACTGGCC GAAGCCGCTT	1620
	GGAATAAGGC CGGTGTGCGT TTGTCTATAT GTTATTTCC ACCATATTGC CGTCTTTGG	1680
55	CAATGTGAGG GCCCCGAAAC CTGGCCCTGT CTTCTTGACG AGCATTCTA GGGGTCTTTC	1740
	CCCTCTCGCC AAAGGAATGC AAGGTCTGTT GAATGTCGTG AAGGAAGCAG TTCCTCTGGA	1800
60	AGCTTCTTGA AGACAAACAA CGTCTGTAGC GACCCTTGC AGGCAGCGGA ACCCCCCACC	1860
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	ACAACCCAG TGCCACGTTG TGAGTTGGAT AGTTGTGGAA AGAGTCAAAT GGCTCTCCTC	1980
65	AAGCGTATTC AACAAAGGGC TGAAGGATGC CCAGAAGGTA CCCCCATTGTA TGGGATCTGA	2040

	TCTGGGGCCT CGGTGCACAT GCTTTACATG TGTTTAGTCG AGGTTAAAAA AACGTCTAGG	2100
	CCCCCGAAC CACGGGGACG TGGTTTCCCT TTGAAAACA CGATAACGGGA TCCACCGGTC	2160
5	GCCACCATGG GTAAAGGAGA AGAACTTTTC ACAGGAGTTG TCCCAATTCT TGTTGAATTA	2220
	GATGGTGATG TTAATGGGCA CAAATTTCT GTCAGTGGAG AGGGTGAAGG TGATGCAACA	2280
10	TACGGAAAAC TTACCCCTAA ATTTATTTCG ACTACTGGAA AACTACCTGT TCCATGGCCA	2340
	ACACTTGTCA CTACTTTCAC TTATGGTGT CAATGCTTT CAAGATAACCC AGATCATATG	2400
	AAACGGCATG ACTTTTCAA GAGTGCCATG CCCGAAGGTT ATGTACAGGA AAGAACTATA	2460
15	TTTTCAAAG ATGACGGGAA CTACAAGACA CGTGCAGAAG TCAAGTTGA AGGTGATACC	2520
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20	CACAAATTGG AATACAACTA TAACTCACAC AATGTATACA TCATGGCAGA CAAACAAAAG	2640
	AATGGAACCA AAGTTAACTT CAAAATTAGA CACAACATTG AAGATGGAAG CGTTCAACTA	2700
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25	CATTACCTGT CCACACAATC TGCCCTTCG AAAGATCCC ACGAAAAGAG AGACCACATG	2820
	GTCTTCTTG AGTTTGTAAAC AGCTGCTGGG ATTACACATG GCATGGATGA ACTATACAAG	2880
	TCCGGATCTA GATAACTGTA TCGATGGATC CGAAGGCGGG GACAGCAGTG CAGTGGTGG	2940
30	CAGAAAGCAA GTGATCTAGG CCAGCAGCCT CCCTAAAGGG ACTTCAGCCC ACAAGCCAA	3000
	ACTTGTGGCT TTAATACAAG CTCTGTAAAT GGTAAAAAAA AAAAGTCTA CACGGACAGC	3060
35	AGGTATGCTC TTGCCACTGT ACAGAGCAAT ATACAGACAA AGAGAACTGT TGACATCTGC	3120
	AGAGAAAGAC CTAAGATGCT GTGGCTAAAAA GAAATCAGAT GGCAAATCTA ACCGCCAGG	3180
	CATCCTAAAG AGCAATGATC CTGACAGTCT GAAGACTATC AAGTTATAGA CAAATTAAGA	3240
40	CTGGTAAAAA AAACCTGTA TAAAATAGTA AAAACTGAAA AAAGAAAACT AGTCCTCTCA	3300
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	AATACCTTCT AGTTTTGTG AACGTTCTCA AGATGGATAA AAGCTTTCC TTGTAAAACG	3420
45	AGACTGATCA GATAGTCATC AAGAAGATTG TAAAGAAAAA TTTCCAAGG TTCGGAGTGC	3480
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50	CCAAGTATTT AGAGGTCAAA TGAAAATTCC ATTGTGTGTA CAGACCTCAG AGCTCAGGAA	3600
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55	GCACAGACTT ACTTGGTACT CCTTCCCCCTT GCCCTATTTA GAACTGAGAA TACTCCCTCT	3720
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	ATGATGTGTT TAAACCTATG TTGTTATAAT AATGATCTAT ATGTTAAGTT AAAAGGCTTG	3840
60	CAGGTGGTGC AGAAAGAAGT CTGGTCACAA CTGGCTACAG TGAACAAGCT GGGTACCCCA	3900
	AGGACATCTT ACCAGTTCCA GCCAGAGATC TGATCTACGA TCCCCGGGTC GACCCGGGTC	3960
	GACCTGTGG AATGTGTGTC AGTTAGGGTG TGGAAAGTCC CCAGGCTCCC CAGCAGGCAG	4020
65	AAGTATGCAA ACCATGCATC TCAATTAGTC AGCAACCAGG TGTGGAAAGT CCCCCAGGCTC	4080

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5	CCTAACTCCG CCCATCCCGC CCCTAACTCC GCCCAGTTCC GCCCATTCTC CGCCCCATGG	4200
	CTGACTAATT TTTTTTATTT ATGCAGAGGC CGAGGCCGCC TCGGCCTCTG AGCTATTCCA	4260
	GAAGTAGTGA GGAGGCTTTT TTGGAGGCCT AGGCTTTGC AAAAAGCTTC ACGCTGCCGC	4320
10	AAGCACTCAG GGCGCAAGGG CTGCTAAAGG AAGCGGAACA CGTAGAAAGC CAGTCCGCAG	4380
	AAACGGTGCT GACCCCGGAT GAATGTCAAG TACTGGGCTA TCTGGACAAG GGAAAACGCA	4440
15	AGCGCAAAGA GAAAGCAGGT AGCTTGCAGT GGGCTTACAT GGCGATAGCT AGACTGGCG	4500
	GTTTTATGGA CAGCAAGCGA ACCGGAATTG CCAGCTGGGG CGCCCTCTGG TAAGGTTGGG	4560
	AAGCCCTGCA AAGTAAACTG GATGGCTTTC TTGCCGCCAA GGATCTGATG GCGCAGGGGA	4620
20	TCAAGATCTG ATCAAGAGAC AGGATGAGGA TCGTTCGCA TGATTGAACA AGATGGATTG	4680
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25	ACAATCGGCT GCTCTGATGC CGCCGTGTTG CCGCTGTCAG CGCAGGGGCCG CCCGGTTCTT	4800
	TTTGTCAAGA CCGACCTGTC CGGTGCCCTG AATGAACACTGC AGGACGAGGG AGCGCGGCTA	4860
	TCGTGGCTGG CCACGACGGG CGTTCCCTGCG TCGACGTTGT CACTGAAGCG	4920
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	GCTCCTGCCG AGAAAAGTATC CATCATGGCT GATGCAATGC GGCAGCTGCA TACGCTTGAT	5040
	CCGGCTACCT GCCCATTGCA CCACCAAGCG AAACATCGCA TCGAGCGAGC ACGTACTCGG	5100
35	ATGGAAGCCG GTCTTGTGCA TCAGGATGAT CTGGACGAAG AGCATCAGGG GCTCGGCCA	5160
	GCCGAACGT TCGCCAGGCT CAAGGCGCGC ATGCCCGACG GCGAGGATCT CGTCGTGACC	5220
40	CATGGCGATG CCTGCTTGCC GAATATCATG TGAAAATG GCGCTTTTC TGGATTCATC	5280
	GACTGTGGCC GGCTGGGTGT GGCAGGACGC TATCAGGACA TAGCGTTGGC TACCGTGAT	5340
	ATTGCTGAAG AGCTTGGCGG CGAATGGGCT GACCGCTTCC TCGTGCTTTA CGGTATCGCC	5400
45	GCTCCCGATT CGCAGCGCAT CGCCTTCTAT CGCCTTCTTG ACGAGTTCTT CTGAGCGGG	5460
	CTCTGGGGTT CGAAATGACC GACCAAGCGA CGCCCAACCT GCCATCACGA GATTCGATT	5520
50	CCACCGCCGC CTTCTATGAA AGGTTGGCT TCGGAATCGT TTTCCGGGAC GGAATTCGTA	5580
	ATCTGCTGCT TGCAAACAAA AAAACCACCG CTACCAGCGG TGGTTGTTT GCGGGATCAA	5640
	GAGCTACCAA CTCTTTTCC GAAGGTAACT GGCTTCAGCA GAGCGCAGAT ACCAAATACT	5700
55	GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTCAAGA ACTCTGTAGC ACCGCCCTACA	5760
	TACCTCGCTC TGCTAACCTC GTTACCAAGTG GCTGCTGCCA GTGGCGATAA GTCGTGTCTT	5820
60	ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCAGC AGCGGTGGGG CTGAACGGGG	5880
	GGTTCGTGCA CACAGCCCAG CTTGGAGCGA ACGACCTACA CCGAACTGAG ATACCTACAG	5940
	CGTGAGCATT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA AGGCGGACAG GTATCCGGTA	6000
65	AGCGGCAGGG TCGGAACAGG AGAGCGCACG AGGGAGCTTC CAGGGGGAAA CGCCTGGTAT	6060

CTTTATAGTC CTGTCGGGTT TCGCCACCTC TGACTTGAGC GTCGATTTT GTGATGCTCG 6120  
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 5 CCTGGCCCTA TTATTGGGTG GACTAACCAT GGGGGAAATT GCCGCTGGAA TAGGAACAGG 6240  
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 10 TCTCAGGGAG GTTGAAAAAT CAATCTCTAA CCTAGAAAAG TCTCTCACCT CCCTGTCTGA 6360  
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 15 CATGGCCAAA TTGAGAGAGA GGCTTAATCA GAGACAGAAA CTGTTTGAGT CAACTCAAGG 6540  
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 20 GGGACCCCTC ATTGTACTCC TAATGATTTT GCTCTTCGGA CCCTGCATTC TTAATCGATT 6660  
 AGTCCAATTG TTAAAGACAA GGATATCAGT GGTCCAGGCT CTAGTTTGA CTCAACAATA 6720  
 TCACCAAGCTG AAGCCTATAG AGTACGAGCC ATAGATAAAA TAAAAGATTT TATTTAGTCT 6780  
 25 CCAGAAAAAG GGGGG 6795  
 (2) INFORMATION FOR SEQ ID NO:23:  
 (i) SEQUENCE CHARACTERISTICS:  
 30 (A) LENGTH: 9093 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: DNA (genomic)  
 35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:  
 40 AATGAAAGAC CCCACCTGTA GGTTTGGCAA GCTAGCTTAA GTAACGCCAT TTTGCAAGGC 60  
 ATGGAAAAAT ACATAACTGA GAATAGAGAA GTTCAGATCA AGGTCAGGAA CAGATGGAAC 120  
 45 AGCTGAATAT GGGCCAAACAA GGATATCTGT GGTAAGCAGT TCCCTGCCCG GCTCAGGGCC 180  
 AAGAACAGAT GGAACAGCTG AATATGGGCC AACAGGATA TCTGTGGTAA GCAGTTCTG 240  
 50 CCCCGGCTCA GGGCCAAGAA CAGATGGTCC CCAGATGCGG TCCAGCCCTC AGCAGTTCT 300  
 AGAGAACCAT CAGATGTTTC CAGGGTGCCTC CAAGGACCTG AAATGACCCCT GTGCCCTTATT 360  
 TGAACTAACCAATCAGTTG CTTCTCGCTT CTGTTCGCGC GCTTCTGCTC CCCGAGCTCA 420  
 55 ATAAAAGAGC CCACAACCCC TCACTCGGGG CGCCAGTCCT CCGATTGACT GAGTCGCCCG 480  
 GGTACCCGTG TATCCAATAA ACCCTCTTGC AGTTGCATCC GACTTGTGGT CTCGCTGTT 540  
 CTTGGGAGGG TCTCCTCTGA GTGATTGACT ACCCGTCAGC GGGGGTCTTT CATTGGGGG 600  
 60 CTCGTCGGG ATCGGGAGAC CCCTGCCAG GGACCACCGA CCCACCACCG GGAGGTAAGC 660  
 TGGCCAGCAA CTTATCTGTG TCTGTCCGAT TGTCTAGTGT CTATGACTGA TTTTATGCGC 720  
 65 CTGCGTCGGT ACTAGTTAGC TAACTAGCTC TGTATCTGGC GGACCCGTGG TGGAACGTGAC 780

	GAGTCGGAA CACCCGGCCG CAACCCTGGG AGACGTCCC GGAGGAACAG GGGAGGATCA	840
	GGGACGCCCTG GTGGACCCCT TTGAAGGCCA AGAGACCATT TGGGGTTGCG AGATCGTGGG	900
5	TTCGAGTCCC ACCTCGTGCC CAGTTGCGAG ATCGTGGGTT CGAGTCCCAC CTCGTGTTT	960
	GTTGCGAGAT CGTGGGTTCG AGTCCCACCT CGCGCTGCGT CACGGGATCG TGGGTTCGAG	1020
10	TCCCACCTCG TGTTTGTG CGAGATCGT GGTCGAGTC CCACCTCGCG TCTGGTCACG	1080
	GGATCGTGGG TTGAGTCCC ACCTCGTGCAG GAGGGTCTCA ATTGGCCGGC CTTAGAGAGG	1140
	CCATCTGATT CTTCTGGTTT CTCTTTTGT CTTAGTCTCG TGTCCGCTCT TGTTGTGACT	1200
15	ACTGTTTTTC TAAAAATGGG ACAATCTGTG TCCACTCCCC TTTCTCTGAC TCTGGTCTG	1260
	TCGCTTGGTA ATTTTGTGTTG TTTACGTTG TTTTGTGAG TCGTCTATGT TGTCTGTTAC	1320
20	TATCTTGTGTT TTGTTTGTGG TTTACGGTTT CTGTTGTGTT CTTGTGTGTC TCTTTGTGTT	1380
	CAGACTTGGG CTGATGACTG ACGACTGTTT TTAAGTTATG CCTTCTAAA TAAGCCTAAA	1440
	AATCCTGTCA GATCCCTATG CTGACCACTT CCTTCAGAT CAACAGCTGC CCTTACGTAT	1500
25	CGATGGATCC CTCGACTAAC TAATAGCCCA TTCTCCAAGG TCGAGCGGGG TCAATTCCGC	1560
	CCCCCCCTTA ACGTTACTGG CGGAAGCCGC TTGGAATAAG GCCGGTGTGC GTTTGTCTAT	1620
30	ATGTTATTTT CCACCATATT GCCGTCTTTT GGCAATGTGA GGGCCCGGAA ACCTGGCCCT	1680
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35	GCGACCCTTT GCAGGCAGCG GAACCCCCCA CCTGGCGACA GGTGCCTCTG CGGCCAAAAG	1860
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	GCCCAGAAGG TACCCCATTG TATGGATCT GATCTGGGTC CTCGGTGCAC ATGCTTTACA	2040
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45	CTTTGAAAAAA CACGATAATA ATCATGGCG CGGATCCCCT CGTTTACAA CGTCGTGACT	2160
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	GGCGTAATAG CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCCGC AGCCTGAATG	2280
50	GCGAATGGCG CTTTGCCTGG TTTCCGGCAC CAGAAGCGGT GCCGGAAAGC TGGCTGGAGT	2340
	GCGATCTTCC TGAGGCCGAT ACTGTCGTGCG TCCCCCTCAA CTGGCAGATG CACGGTTACG	2400
55	ATGCGCCCAT CTACACCAAC GTAACCTATC CCATTACGGT CAATCCGCCG TTTGTCTCCA	2460
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60	AAGGCCAGAC CGGAATTATT TTTGATGGCG TTAACTCGGC GTTTCATCTG TGGTGCAACG	2580
	GGCGCTGGGT CGGTTACGGC CAGGACAGTC GTTGCCTGTC TGAATTGAC CTGAGCGCAT	2640
	TTTTACGCCGCG CGGAGAAAAC CGCCTCGCGG TGATGGTGCT GCGTTGGAGT GACGGCAGTT	2700
65	ATCTGGAAGA TCAGGATATG TGGCGGATGA GCGGCATTTT CCGTGACGTC TCGTTGCTGC	2760

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	GCCGCGCTGT ACTGGAGGCT GAAGTTCAGA TGTGCGCGA GTTGCCTGAC TACCTACGGG	2880
5	TAACAGTTTC TTTATGGCAG GGTGAAACGC AGGTCGCCAG CGGCACCGCG CCTTTCGGCG	2940
	GTGAAATTAT CGATGAGCGT GGTGGTTATG CCGATCGCGT CACACTACGT CTGAACGTCG	3000
10	AAAACCCGAA ACTGTGGAGC GCCGAAATCC CGAACCTCTA TCGTGCGGTG GTTGAACGTGC	3060
	ACACCGCCGA CGGCACGCTG ATTGAAGCAG AAGCCTGCGA TGTCGGTTTC CGCGAGGTGC	3120
	GGATTGAAAA TGGTCTGCTG CTGCTGAACG GCAAGCCGTT GCTGATTGCA GGCGTTAACCC	3180
15	GTCACGAGCA TCATCCTCTG CATGGTCAGG TCATGGATGA GCAGACGATG GTGCAGGATA	3240
	TCCTGCTGAT GAAGCAGAAC AACTTTAACG CCGTGCCTG TTCCGATTAT CGAACCATC	3300
20	CGCTGTGGTA CACGCTGTGC GACCGCTACG GCCTGTATGT GGTGGATGAA GCCAATATTG	3360
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25	GGTCGCTGGG GAATGAATCA GGCCACGGCG CTAATCACGA CGCGCTGTAT CGCTGGATCA	3540
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30	CGAAATGGTC CATCAAAAAA TGGCTTCGC TACCTGGAGA GACGCGCCCG CTGATCCTTT	3720
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35	TTCGTCAGTA TCCCCGTTA CAGGGCGGCT TCGTCTGGGA CTGGGTGGAT CAGTCGCTGA	3840
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	ACCGGATTGA TGTTAGTGGT CAAATGGCGA TTACCGTTGA TGTTGAAGTG GCGAGCGATA	4740
65	CACCGCATCC GGCGCGGATT GGCCTGAACG GCCAGCTGGC GCAGGTAGCA GAGCGGGTAA	4800

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25	GTTATAGACA ATTAAGACT GGTAAAAAAA ACCCTGTATA AAATAGTAAA AACTGAAAAA	5580
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	ATGCCATCAC TGTCTAAAT GATGTGTTTA AACCTATGTT GTTATAATAA TGATCTATAT	6120
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50	AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG	6360
	TGGAAAGTCC CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC	6420
55	AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACCTCCGC CCAGTTCCGC	6480
	CCATTCTCCG CCCCCATGGCT GACTAATTT TTTTATTAT GCAGAGGCCG AGGCCGCCTC	6540
	GGCCTCTGAG CTATTCCAGA AGTAGTGAGG AGGCTTTTT GGAGGCCTAG GCTTTGCAA	6600
60	AAAGCTTCAC GCTGCCGCAA GCACTCAGGG CGCAAGGGCT GCTAAAGGAA GCGGAACACG	6660
	TAGAAAGCCA GTCCGCAGAA ACGGTGCTGA CCCCCGATGA ATGTCAGCTA CTGGCCTATC	6720
65	TGGACAAGGG AAAACGCAAG CGCAAAGAGA AAGCAGGTAG CTTGCAGTGG GCTTACATGG	6780

	CGATAGCTAG ACTGGGCGGT TTTATGGACA GCAAGCGAAC CGGAATTGCC AGCTGGGCG	6840
	CCCTCTGGTA AGGTTGGAA GCCCTGCAA GTAAACTGGA TGGCTTCTT GCCGCCAAGG	6900
5	ATCTGATGGC GCAGGGGATC AAGATCTGAT CAAGAGACAG GATGAGGATC GTTTCGCATG	6960
	ATTGAACAAG ATGGATTGCA CGCAGGTTCT CCGGGCGCTT GGGTGGAGAG GCTATTCGGC	7020
10	TATGACTGGG CACAACAGAC AATCGGCTGC TCTGATGCCG CCGTGTCCG GCTGTCAGCG	7080
	CAGGGCGCC CGGTTCTTT TGTCAAGACC GACCTGTCCG GTGCCCTGAA TGAACTGCAG	7140
	GACGAGGCAG CGCGGCTATC GTGGCTGCC ACACGGGCG TTCCCTGCCG AGCTGTGCTC	7200
15	GACGTTGTCA CTGAAGCGGG AAGGGACTGG CTGCTATTGG GCGAAGTGCC GGGGCAGGAT	7260
	CTCCTGTCAT CTCACCTTGC TCCTGCCAG AAAGTATCCA TCATGGCTGA TGCAATGCGG	7320
20	CGGCTGCATA CGCTTGTATCC GGCTACCTGC CCATTCGACC ACCAACGCAA ACATCGCATC	7380
	GAGCGAGCAC GTACTCGGAT GGAAGCCGGT CTTGTCGATC AGGATGATCT GGACGAAGAG	7440
	CATCAGGGGC TCGCGCCAGC CGAACCTGTC GCCAGGCTCA AGGCGCGCAT GCCCGACGGC	7500
25	GAGGATCTCG TCGTGACCCA TGGCGATGCC TGCTTGCCGA ATATCATGGT GGAAAATGGC	7560
	CGCTTTCTG GATTCATCGA CTGTGCCGG CTGGGTGTGG CGGACCGCTA TCAGGACATA	7620
	GCGTTGGCTA CCCGTGATAT TGCTGAAGAG CTTGGCGGCG AATGGGCTGA CCGCTTCCTC	7680
30	GTGCTTTACG GTATGCCGC TCCCGATTG CAGCGCATCG CCTTCTATCG CCTTCTTGAC	7740
	GAGTTCTTCT GAGCGGGACT CTGGGGTTCG AAATGACCGA CCAAGCGACG CCCAACCTGC	7800
35	CATCACGAGA TTTCGATTCC ACCGCCGCCT TCTATGAAAG GTTGGGCTTC GGAATCGTT	7860
	TCCGGGACGG AATTCTGTAAT CTGCTGCTTG CAAACAAAAA AACCAACCGCT ACCAGCGGTG	7920
	GTGGTTTGCG CGGATCAAGA GCTACCAACT CTTTTCCGA AGGTAACTGG CTTCAGCAGA	7980
40	GCGCAGATAAC CAAATACTGT CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC	8040
	TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAAGTGGC TGCTGCCAGT	8100
45	GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG	8160
	CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC GACCTACACC	8220
	GAACTGAGAT ACCTACAGCG TGAGCATTGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG	8280
50	GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA	8340
	GGGGGAAACG CCTGGTATCT TTATAGTCCT GTGGGGTTTC GCCACCTCTG ACTTGAGCGT	8400
55	CGATTTTGT GATGCTCGTC AGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCCAG	8460
	ATGCGCCGCC TCGAGAACCC TGGCCCTATT ATTGGGTGGA CTAACCATGG GGGGAATTGC	8520
	CGCTGGAATA GGAACAGGGA CTACTGCTCT AATGCCACT CAGCAATTCC AGCAGCTCCA	8580
60	AGCCGCAGTA CAGGATGATC TCAGGGAGGT TGAAAAATCA ATCTCTAACC TAGAAAAGTC	8640
	TCTCACTTCC CTGTCTGAAG TTGTCTTACA GAATCGAAGG GGCCTAGACT TGTTATTCT	8700
65	AAAAGAAGGA GGGCTGTGTG CTGCTCTAAA AGAAGAATGT TGCTTCTATG CGGACCACAC	8760
	AGGACTAGTG AGAGACAGCA TGGCCAAATT GAGAGAGAGG CTTAATCAGA GACAGAAACT	8820

	GTTTGAGTCA ACTCAAGGAT GGTTTGAGGG ACTGTTAAC AGATCCCCCTT GGTTTACAC	8880
5	CTTGATATCT ACCATTATGG GACCCCTCAT TGTACTCCTA ATGATTTGC TCTTCGGACC	8940
	CTGCATTCTT AATCGATTAG TCCAATTGT TAAAGACAGG ATATCAGTGG TCCAGGCTCT	9000
	AGTTTGACT CAACAATATC ACCAGCTGAA GCCTATAGAG TACGAGCCAT AGATAAAATA	9060
10	AAAGATTTA TTTAGTCTCC AGAAAAAGGG GGG	9093

## (2) INFORMATION FOR SEQ ID NO:24:

15 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 46 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: DNA (genomic)

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GACTAACCTT GATTCCCTGG AGGCGGGGGT CTTTCATTTG GGGGCT

46

## (2) INFORMATION FOR SEQ ID NO:25:

30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 4834 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: DNA (genomic)

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TGAAGAAATAA AAAATTACTG GCCTCTTGTG AGAACATGAA CTTCACCTC GGAGCCCACC	60
45 CCCTCCCATC TGAAAACAT ACTTGAGAAA AACATTTCT GGAACAACCA CAGAATGTTT	120
CAACAGGCCA GATGTATTGC CAAACACAGG ATATGACTCT TTGGTTGAGT AAATTGTGG	180
50 TTGTTAAACT TCCCCTATTC CCTCCCCATT CCCCTCCCA GTTTGTGGTT TTTTCCCTTA	240
AAAGCTTGTG AAAAATTGAA GTCGTCGTCG AGACTCCTCT ACCCTGTGCA AAGGTGTATG	300
AGTTTCGACC CCAGAGCTCT GTGTGCTTTC TGTTGCTGCT TTATTCGAC CCCAGAGCTC	360
55 TGGTCTGTGT GCTTTCATGT CGCTGCTTTA TTAAATCTTA CCTTCTACAT TTTATGTATG	420
GTCTCAGTGT CTTCTGGGT ACGCAGGCTGT CCCGGGACTT GAGTGTCTGA GTGAGGGTCT	480
60 TCCCTCGAGG GTCTTCATT TGGTACATGG GCCGGGAATT CGAGAACCTT TCATTGGTG	540
CATTGGCCGG GAATTGAAA ATCTTCATT TGGTGCATTG GCCGGGAAAC AGCGCGACCA	600
CCCAGAGGTC CTAGACCCAC TTAGAGGTAAG GATTCTTGT TCTGTTTGG TCTGATGTCT	660
65 GTGTTCTGAT GTCTGTGTTG TGTTCTAAG TCTGGTGCAG TCGCAGTTTC AGTTTGCGG	720

	ACGCTCAGTG AGACCGCGCT CCGAGAGGGA GTGCCGGGTG GATAAGGATA GACGTGTCCA	780
	GGTGTCCACC GTCCGTTCGC CCTGGGAGAC GTCCCAGGAG GAACAGGGGA GGATCAGGGA	840
5	CGCCTGGTGG ACCCCTTGA AGGCCAAGAG ACCATTGGG GTTGCAGAGAT CGTGGGTTCG	900
	AGTCCCACCT CGTGCCCCAGT TGCGAGATCG TGTTTCGAG TCCACCTCG TGTTTGTTG	960
10	CGAGATCGTG GGTCGAGTC CCACCTCGCG TCTGGTCACG GGATCGTGGG TTCGAGTCCC	1020
	ACCTCGTGT TTGTTGCGAG ATCGTGGGTT CGAGTCCCAC CTCGCGTCTG GTCACGGGAT	1080
	CGTGGGTTCG AGTCCCACCT CGTGAGGAG GTCTCAATTG GCCGGCCTTA GAGAGGCCAT	1140
15	CTGATTCTTC TGGTTCTCT TTTTGTCTTA GTCTCGTGT CGCTCTTGTG GTGACTACTG	1200
	TTTTCTAAA AATGGGACAA TCTGTGTCCA CTCCCCTTTC TCTGACTCTG GTTCTGTGCG	1260
20	TTGGTAATTT TGTTGTTTA CGTTTGTGTT TGTGAGTCGT CTATGTTGTC TGTTACTATC	1320
	TTGTTTTGT TTGTGGTTA CGGTTCTGT GTGTGTCTTG TGTGTCTCTT TGTGTTCAAGA	1380
	CTTGGACTGA TGACTGACGA CTGTTTTAA GTTATGCCCTT CTAAAATAAG CCTAAAAATC	1440
25	CTGTCAGATC CCTATGCTGA CCACCTCCTT TCAGATCAAC AGCTGCCCTG CCTCCCACTC	1500
	CAACTCCAGA GAGCAGCCAG CGGGTCACAG TGGTCCCGCC CATGAACCTG GAGCCTAGGG	1560
	AAAAATGAGC TCGGAAATCC GGAGCAAATG AGGAGTGGTC CCTGAGAAGT CAGTGGCCTA	1620
30	AATGTTGTGG CTGCTGAAGC AAAAGAAGAG GAGGCTGTTC GAGTAGCCGG CCAAGAGCGC	1680
	CGCGGGTTCC CAGGCAGCTT CTCATTCCCC TGTCCCTCCC ATCCCGTCTC TTGTTAACAG	1740
35	AAAAACTGCT TTCACTTTGA GATATGAGTG GCCCGATACA GCCAGCTGT AGAGCTGTAC	1800
	TCCCTTCCCT GCCCCACGTG TTTTCTCTTC TCAGGGGACC CCTCCCTGAG CTGCTGGCAG	1860
	TGAGTCTGTT CTAAGCTCCA GTGAGGGAGG CATCCGCCA CTTGGGGCTT CTGTCCAAGG	1920
40	TAAGGAGCAC CTGTGAGTCT AACTGCCAGG CTCTGATGGG GGTCTCGTCT CTGTGGACT	1980
	AGAAAGTGTG CCAACAATCT GACCAAGGTA ACAGGAAGTT AAGACAAAGA CAGAGACCAA	2040
45	AGTCAGAACATC AGAGCTGTGC TGTGAGACAA AAAGATAAAA AAAATAAAAT GCTGGCCACA	2100
	AAAGTCAGGA AAACTAGAAA ACTTAGATAG TACCTGGCAA CAAAAGAAAG CTTTTGGCTA	2160
	AAGATCAACG TGTATACTGT AAAGAAAATG AGCACTGGGT GAGAGACTGC CCCAACAAAA	2220
50	AGAAAGAGGAG CCCCCCTCAT GACCAAAACCC TTCACCTGTT CGTGGCTAAA AGTAAAGAGA	2280
	TAACAAAAGG GGTGCTAACCA CAGAAGCTGA GTCCTTAAAA GAGTCCGGTG GCCTACCTGT	2340
55	TGAAGCAGCT AAAAAAGAGA CTGTGTTCA TACTCCTCCA CTGACCAGTG CAAACAAAGC	2400
	TAAAAAAGTTC CTGGGCAGTG CGGGCTTTG CAGATTGTGG ATTCCAGGTT TTGCTGAGTT	2460
	AAAGAGATAA ACAGCCCTTC GTATAGAAAA ATAAAAAAACA ACCTTGGATG TCCTTGGATG	2520
60	CTATTGAGAC TGCCCTAATG TTGTCCCCAG CTATGGGACT CCTAGATGTG ACTGAGAACAA	2580
	AAGGTATTGC CAAAGAAGTT CTTACTCAGA GATTGGGACC CTGAAAAAGA CCTGTGGCAT	2640
	ACTTGTAAAGA AATTAGACCT GGTGGCTGTA AGATGGCCTG CTTGTCTGCA CATAGTGGCT	2700
65	TCTGGTCAAG GACGCAGATA AATTGACTCT GAGACAAAAC TTGGCACATG TCCTAGAAAG	2760

	TGTGGTCAG	CCCCCATGAC	CGATGGCTGA	CTAACGCTCT	TGAAAACATT	ATCCAACGT	2820
5	TCCCCTGACC	GATGGACACA	TTGTCAGAGC	TTTTTTGAC	TGAACGAGTG	ACCTTCGCTC	2880
	CCCCTGCTAT	CCTCGATCTC	ACTACTGCCT	GAGACTTCAC	CTACTCATCA	TTGTGCTGAC	2940
	ATTCTGGCAG	AAGAAACTCA	TACTCGAAAT	GATCTGAAGG	ATCAGATCAC	CCTTGGCCTG	3000
10	AGAGTTGAG	CTGGTACACG	GATGGCAGTA	GCCTGGAGGT	TAAGGGTAAG	CGGAAGGC GG	3060
	GGACAGCAGT	GCAGTGGTGG	ACAGAAAGCA	AGTGAATCTAG	GCCAGCAGCC	TCCCTAAAGG	3120
15	GACTTCAGCC	CACAAAGCCA	AACTTGTGGC	TTTAATACAA	GCTCTGTAAA	TGGTAAAAAA	3180
	AAAAAAAGTCT	ACACGGACAG	CAGGTATGCT	CTTGCCACTG	TACAGAGCAA	TATACAGACA	3240
	AAGAGAACTG	TTGACATCTG	CAGAGAAAGA	CCTAAGATGC	TGTGGCTAAA	AGAAATCAGA	3300
20	TGGCAAATCT	AACCGCCAG	GCATCCTAAA	GAGCAATGAT	CCTGACAGTC	TGAAGACTAT	3360
	CAAGTTATAG	ACAAATTAAG	ACTGGTAAAAA	AAAACCTGT	ATAAAATAGT	AAAAACTGAA	3420
	AAAAGAAAAC	TAGTCCTCTC	ATGAGAAGAC	AGACCTGACA	TCTACTGAAA	AATAGACTTT	3480
25	ACTGGAAAAAA	ATATGTGTAT	GAATACCTTC	TAGTTTTGT	GAACGTTCTC	AAGATGGATA	3540
	AAAGCTTTTC	CTTGTAACAC	GAGACTGATC	AGATAGTCAT	CAAGAAGATT	GTTAAAGAAA	3600
30	ATTTTCCAAG	GTTCGGAGTG	CCAAAAGCAA	TAGTGTAGA	TAATGGTCCT	GCCTTGTG	3660
	CCCAGGTAAG	TCAGGGTGTG	GCCAAGTATT	TAGAGGTCAA	ATGAAAATTC	CATTGTGTGT	3720
	ACAGACCTCA	GAGCTCAGGA	AAGATAAAAA	AGAATAAATA	AAACTCTAAA	CAGACCTGA	3780
35	CAAAATTAAT	CCTAGAGACT	GGCACAGACT	TACTGGTAC	TCCTTCCCCT	TGCCCTATTT	3840
	AGAAACTGAGA	ATACTCCCTC	TTGATTGGT	TTTACTCTTT	TTAAGATCCT	TTATGGGGCT	3900
40	CCTATGCCAT	CACTGTCTTA	AATGATGTGT	TTAACCTAT	GTGTTATAA	TAATGATCTA	3960
	TATGTTAAGT	TTAAAGGCTT	GCAGGTGGTG	CAGAAAGAAG	TCTGGTCACA	ACTGGCTACA	4020
	GTGAACAAGC	TGGGTACCCC	AAGGACATCT	TACCAAGTCC	AGCCAGAGAT	CTGATCTACG	4080
45	TACACCTGCG	TCATGCTGAG	ACCCCTCAAGC	CTCACTAAAA	GGGTCCCTGC	CTAGTTCTGT	4140
	TTACTAATCT	GCCTTATTCT	GTGTTGTTC	CCATGTTAAA	GATAGAGTAA	ATGCAGTATT	4200
	CTCCACATAG	AGATATAGAC	TTCTGAAATT	CTAAGATTAG	AATTATTTAC	AAGAAGAAGT	4260
50	GGGGAATGAA	GAATAAAAAAA	TTACTGGCCT	CTTGTGAGAA	CATGAACCTT	CACCTCGGAG	4320
	CCCACCCCT	CCCACATCTGGA	AAACATACTT	GAGAAAAACA	TTTCTGGAA	CAACCACAGA	4380
55	ATGTTCAAC	AGGCCAGATG	TATTGCCAAA	CACAGGATAT	GACTCTTGG	TTGAGTAAAT	4440
	TTGTGGTTGT	TAAACTTCCC	CTATTCCCTC	CCCATTCCCC	CTCCCAGTT	GTGGTTTTT	4500
60	CCTTTAAAAG	CTTGTGAAAAA	ATTTGAGTCG	TCGTCGAGAC	TCCTCTACCC	TGTGCAAAGG	4560
	TGTATGAGTT	TCGACCCAG	AGCTCTGTGT	GCTTCTGTT	GCTGCTTTAT	TTCGACCCCA	4620
	GAGCTCTGGT	CTGTGTGCTT	TCATGTCGCT	GCTTTATTAA	ATCTTACCTT	CTACATTTA	4680
65	TGTATGGTCT	CAGTGTCTTC	TTGGGTACGC	GGCTGTCCCG	GGACTTGAGT	GTCTGAGTGA	4740

GGGTCTTCCC TCGAGGGTCT TTCATTTGGT ACATGGGCCG GGAATTGAG AATCTTCAT 4800  
 TTGGTGCATT GGCCGGGAAT TCGAAAATCT TTCA 4834

## 5 (2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 4518 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA (genomic)

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

20	CACCTGACGC GCCCTGTAGC GGCGCATTAAC CGCGGGCGGG TGTGGTGGTT ACGCGCAGCG	60
	TGACCGCTAC ACTTGCCAGC GCCCTAGCGC CCGCTCCTTT CGCTTTCTTC CCTTCCTTTC	120
25	TCGCCACGTT CGCCGGCTTT CCCCGTCAAG CTCTAAATCG GGGGCTCCCT TTAGGGTTCC	180
	GATTTAGTGC TTTACGGCAC CTCGACCCCC AAAAACCTGAA TTAGGGTGAT GGTCACGTA	240
	GTGGGCCATC GCCCTGATAG ACGGTTTTC GCCCTTGAC GTTGGAGTCC ACGTTCTTA	300
30	ATAGTGGACT CTTGTTCCAA ACTGGAACAA CACTCAACCC TATCTGGTC TATTCTTTG	360
	ATTTTATAAGG GATTTGCCG ATTCGGCCT ATTGGTTAAA AAATGAGCTG ATTTAACAAA	420
35	AATTAAACGC GAATTTAAC AAAATATTAA CGCTTACAAT TTACGCGTTA AGATACATTG	480
	ATGAGTTTGG ACAAAACCACA ACTAGAATGC AGTGGAAAAAA ATGCTTTATT TGTGAAATT	540
	GTGATGCTAT TGCTTTATT GTAACCATTAA TAAGCTGCAA TAAACAAGTT AACAAACAACA	600
40	ATTGCATTCA TTTTATGTT CAGGTTCAAGG GGGAGGTGTG GGAGGTTTT TAAAGCAAGT	660
	AAAACCTCTA CAAATGTGGT ATGGCTGATT ATGATCATGA ACAGACTGTG AGGACTGAGG	720
45	GGCCTGAAAT GAGCCTTGGG ACTGTGAATC TAAAATACAC AAACAATTAG AATCAGTAGT	780
	TTAACACATT ATACACTTAA AAATTGGATC TCCATTGCC ATTCAAGGTG CGCAACTGTT	840
	GGGAAGGGCG ATCGGTGCGG GCCTCTCGC TATTACGCCA GCTGGCGAAA GGGGGATGTG	900
50	CTGCAAGGCG ATTAAGTTGG GTAAACGCCAG GGTGTTCCCA GTCACGACGT TGTAAAACGA	960
	CGGCCAGTGA ATTGTAATAC GACTCACTAT AGGGCGAATT GGGTACACTT ACCTGGTACC	1020
55	CCACCCGGGT GGAAAATCGA TGGGCCCGCG GCCGCTCTAG AAGTACTCTC GAGAAGCTTT	1080
	TTGAATTCTT TGGATCCACT AGTGTGACCC TGCAGGGCGCG CGAGCTCCAG CTTTTGTTCC	1140
	CTTTAGTGAG GGTTAATTTC GAGCTTGGCG TAATCAAGGT CATAGCTGTT TCCTGTGTGA	1200
60	AATTGTTATC CGCTCACAAT TCCACACAAT ATACGAGCCG GAAGTATAAA GTGTAAAGCC	1260
	TGGGGTGCCT AATGAGTGAG CTAACTCACA GTAATTGCCG CTAGCGGATC TGACGGTTCA	1320
65	CTAAACCAGC TCTGCTTATA TAGACCTCCC ACCGTACACG CCTACCGCCC ATTTGCGTCA	1380
	ATGGGGCGGA GTTGTACGA CATTGGAA AGTCCCGTTG ATTTGGTGC CAAAACAAAC	1440

	TCCCATTGAC	GTCAATGGGG	TGGAGACTTG	GAAATCCCCG	TGAGTCAAAC	CGCTATCCAC	1500
5	GCCCATTGAT	GTACTGCCAA	AACCGCATCA	CCATGGTAAT	AGCGATGACT	AATACTAGA	1560
	TGTACTGCCA	AGTAGGAAAG	TCCCATAAGG	TCATGTACTG	GGCATAATGC	CAGGCGGGCC	1620
	ATTTACCGTC	ATTGACGTCA	ATAGGGGCG	TACTTGGCAT	ATGATACTACT	TGATGTACTG	1680
10	CCAAGTGGGC	AGTTTACCGT	AAATACTCCA	CCCATTGACG	TCAATGGAAA	GTCCCTATTG	1740
	GCGTTACTAT	GGGAACATAC	GTCATTATTG	ACGTCAATGG	GCGGGGGTCG	TTGGGCGGTC	1800
15	AGCCAGGCAG	GCCATTAC	GTAAGTTATG	TAACCGGAA	CTCCATATAT	GGGCTATGAA	1860
	CTAATGACCC	CGTAATTGAT	TACTATTAAT	AACTAATGCA	TGGCGGTAAT	ACGGTTATCC	1920
	ACAGAACATAG	GGGATAACGC	AGGAAAGAAC	ATGTGAGCAA	AAGGCCAGCA	AAAGGCCAGG	1980
20	AACCGTAAAAA	AGGCCGCGTT	GCTGGCGTT	TTCCATAGGC	TCCGCC	TGACGAGCAT	2040
	CACAAAAATC	GACGCTCAAG	TCAGAGGTGG	CGAAACCGA	CAGGACTATA	AAGATACCAG	2100
	GCGTTTCCCC	CTGGAAGCTC	CCTCGTGC	TCTCCTGTT	CGACCCTGCC	GCTTACCGGA	2160
25	TACCTGTCCG	CCTTCTCCC	TTCGGGAAAGC	GTGGCGTTT	CTCATAGCTC	ACGCTGTAGG	2220
	TATCTCAGTT	CGGTGTAGGT	CGTCGCTCC	AAGCTGGCT	GTGTGCACGA	ACCCCCGTT	2280
30	CAGCCCGACC	GCTGCGCCTT	ATCCGGTAAC	TATCGTCTTG	AGTCCAACCC	GGTAAGACAC	2340
	GACTTATCGC	CACTGGCAGC	AGCCACTGGT	AACAGGATTAA	GCAGAGCGAG	GTATGTAGGC	2400
	GGTGCTACAG	AGTTCTTGAA	GTGGTGGCCT	AACTACGGCT	ACACTAGAAG	GACAGTATTT	2460
35	GGTATCTGCG	CTCTGCTGAA	GCCAGTTACC	TTCGGAAAAAA	GAGTTGGTAG	CTCTTGATCC	2520
	GGCAAACAAA	CCACCGCTGG	TAGCGGTGGT	TTTTTGTTT	GCAAGCAGCA	GATTACGCGC	2580
40	AGAAAAAAAG	GATCTCAAGA	AGATCCTTG	ATCTTTCTA	CGGGGTCTGA	CGCTCAGTGG	2640
	AACGAAAAC	CACGTTAAGG	GATTTGGTC	ATGAGATTAT	CAAAAAGGAT	CTTCACCTAG	2700
	ATCCTTTAA	ATTAACAAATG	AAGTTTAAA	TCAATCTAAA	GTATATATGA	GTAAACCTGAG	2760
45	GCTATGGCAG	GGCCTGCCGC	CCCGACGTTG	GCTGCGAGCC	CTGGGCCTTC	ACCCGAACCTT	2820
	GGGGGGTGGG	GTGGGGAAAAA	GGAAGAAACG	CGGGCGTATT	GGCCCCAATG	GGGTCTCGGT	2880
50	GGGGTATCGA	CAGAGTGCCA	GCCCTGGGAC	CGAACCCCGC	GTTTATGAAC	AAACGACCCA	2940
	ACACCGTGCG	TTTATTCTG	TCTTTTATT	GCCGTCTAG	CGCGGGTTCC	TTCCGGTATT	3000
	GTCTCCTTCC	GTGTTTCAGT	TAGCCTCCCC	CTAGGGTGGG	CGAAGAACTC	CAGCATGAGA	3060
55	TCCCCCGCCT	GGAGGATCAT	CCAGCCGGCG	TCCCCGAAAAA	CGATTCCGAA	GCCCCAACCTT	3120
	TCATAGAAGG	CGCGGGTGGG	ATCGAAATCT	CGTGATGGCA	GGTTGGGCGT	CGCTTGGTCG	3180
	GTCATTTCGA	ACCCCAGAGT	CCCGCTCAGA	AGAACTCGTC	AAGAAGGCGA	TAGAAGGCAGA	3240
60	TGCGCTGCGA	ATCGGGAGCG	GCGATACCGT	AAAGCACGAG	GAAGCGGTCA	GCCCATTGCG	3300
	CGCCAAGCTC	TTCAGCAATA	TCACGGGTAG	CCAACGCTAT	GTCCTGATAG	CGGTCCGCCA	3360
65	CACCCAGCCG	GCCACAGTCG	ATGAATCCAG	AAAAGCGGCC	ATTTCACC	ATGATATTG	3420

	GCAAGCAGGC ATGCCATGG GTCACGACGA GATCCTCGCC GTCGGGCATG CTCGCCCTG	3480
	GCCTGGCGAA CAGTTCGGCT GGCGCGAGCC CCTGATGCTC TTCTGTCCAGA TCATCCTGAT	3540
5	CGACAAGACC GGCTTCCATC CGAGTACGTG CTCGCTCGAT GCGATGTTTC GCTTGGTGGT	3600
	CGAATGGGCA GGTAGCCGGA TCAAGCGTAT GCAGCCGCCG CATTGCATCA GCCATGATGG	3660
10	ATACTTCTC GGCAGGAGCA AGGTGAGATG ACAGGAGATC CTGCCCCGGC ACTTCGCCA	3720
	ATAGCAGCCA GTCCCCTTCCC GCTTCAGTGA CAACGTCGAG CACAGCTGCG CAAGGAACGC	3780
	CCGTCGTGGC CAGCCACGAT AGCCGCGCTG CCTCGTCTTG CAGTTCATTC AGGGCACCGG	3840
15	ACAGGTCGGT CTTGACAAAA AGAACCGGGC GCCCCTGCGC TGACAGCGG AACACGGCGG	3900
	CATCAGAGCA GCCGATTGTC TGTTGTGCC AGTCATAGCC GAATAGCCTC TCCACCAAG	3960
	CGGCCCCGAGA ACCTGCGTGC AATCCATCTT GTTCAATCAT GCGAAACGAT CCTCATCCTG	4020
20	TCTCTTGATC GATCTTGCA AAAGCCTAGG CCTCCAAAAA AGCCTCCTCA CTACTTCTGG	4080
	AATAGCTCAG AGGCCGAGGC GGCTCGGCC TCTGCATAAA TAAAAAAAAT TAGTCAGCCA	4140
25	TGGGGCGGAG AATGGGCAGA ACTGGGCAGA GTTAGGGCGG GGATGGCGG AGTTAGGGC	4200
	GGGACTATGG TTGCTGACTA ATTGAGATGC ATGCTTGCA TACTTCTGCC TGCTGGGAG	4260
	CCTGGGGACT TTCCACACCT GGTTGCTGAC TAATTGAGAT GCATGCTTG CATACTTCTG	4320
30	CCTGCTGGGG AGCCTGGGA CTTTCCACAC CCTAACTGAC ACACATTCCA CAGCTGGTTC	4380
	TTTCCGCCTC AGGACTCTTC CTTTTCAAT ATTATTGAAG CATTATCAG GGTTATTGTC	4440
35	TCATGAGCGG ATACATATT GAATGTATT AGAAAATAA ACAAAATAGGG GTTCCCGCGCA	4500
	CATTTCCCCG AAAAGTGC	4518

## (2) INFORMATION FOR SEQ ID NO:27:

- 40       (i) SEQUENCE CHARACTERISTICS:  
           (A) LENGTH: 25 base pairs  
           (B) TYPE: nucleic acid  
           (C) STRANDEDNESS: single  
           (D) TOPOLOGY: linear
- 45       (ii) MOLECULE TYPE: DNA (genomic)

50       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:  
 CTCCACATAG AGATATAGAC TTCTG

25

## 55       (2) INFORMATION FOR SEQ ID NO:28:

- 55       (i) SEQUENCE CHARACTERISTICS:  
           (A) LENGTH: 45 base pairs  
           (B) TYPE: nucleic acid  
           (C) STRANDEDNESS: single  
           (D) TOPOLOGY: linear
- 60       (ii) MOLECULE TYPE: DNA (genomic)

65

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

5 CGATCTTATT AATTAACCTGG AGTTTGAGC CCRMCCCCTC CCATC 45

## (2) INFORMATION FOR SEQ ID NO:29:

10 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 5594 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

20	TGCATTAGTT ATTAATAGTA ATCAATTACG GGGTCATTAG TTCATAGCCC ATATATGGAG	60
	TTCCCGCTTA CATAACTTAC GGTAAATGGC CCCGCTGGCT GACCGCCCAA CGACCCCCGC	120
25	CCATTGACGT CAATAATGAC GTATGTTCCC ATAGTAACGC CAATAGGGAC TTTCCATTGA	180
	CGTCAATGGG TGGAGTATT ACGGTAAACT GCCCACTTGG CAGTACATCA AGTGTATCAT	240
	ATGCCAAGTA CGCCCCCTAT TGACGTCAT GACGGTAAAT GGCCCGCCTG GCATTATGCC	300
30	CAGTACATGA CCTTATGGGA CTTTCCTACT TGGCAGTACA TCTACGTATT AGTCATCGCT	360
	ATTACCATGG TGATGCGGTT TTGGCAGTAC ATCAATGGC GTGGATAGCG GTTTGACTCA	420
	CGGGGATTTC CAAGTCTCCA CCCCATTGAC GTCAATGGGA GTTTGTTTG GCACCAAAAT	480
35	CAACGGGACT TTCCAAAATG TCGTAACAAC TCCGCCCCAT TGACGCAAAT GGGCGGTAGG	540
	CGTGTACGGT GGGAGGTCTA TATAAGCAGA GCTGGTTAG TGAACCGTCA GATCCCGGCC	600
40	AGTCCTCCGA TTGACTGAGT CGCCCGGGTA CCCGTGTATC CAATAAACCC TCTTGCAGTT	660
	GCATCCGACT TGTGGTCTCG CTGTTCTTG GGAGGGTCTC CTCTGAGTGA TTGACTACCC	720
	GTCAGCGGGG GTCTTCATT TGGGGCTCG TCCGGGATCG GGAGACCCCT GCCCAGGGAC	780
45	CACCGACCCA CCACCGGGAG GTAAGCTGGC CAGCACTTA TCTGTGTCTG TCCGATTGTC	840
	TAGTGTCTAT GACTGATTTT ATGCGCCTGC GTCGGTACTA GTTAGCTAAC TAGCTCTGTA	900
50	TCTGGCGGAC CCGTGGTGGA ACTGACGGAGT TCGAACACCC CGGCCGCAAC CCTGGGAGAC	960
	GTCCCAGGAG GAACAGGGGA GGATCAGGGA CGCCTGGTGG ACCCCTTGA AGGCCAAGAG	1020
	ACCATTTGGG GTTGCAGAT CGTGGGTTCG AGTCCCACCT CGTCCCCAGT TGCGAGATCG	1080
55	TGGGTTCGAG TCCCACCTCG TGTTTGTG CGAGATCGTG GGTCGAGTC CCACCTCGCG	1140
	TCTGGTCACG GGATCGTGGG TTTCGAGTCCC ACCTCGTGT TTGTTGCGAG ATCGTGGGTT	1200
60	CGAGTCCCCAC CTCGCGTCTG GTCACGGGAT CGTGGGTTCG AGTCCCACCT CGTGCAGAGG	1260
	GTCTCAATTG GCCGGCCTTA GAGAGGCCAT CTGATTCTTC TGGTTCTCT TTTTGTCTTA	1320
	GTCTCGTGTGTC CGCTCTTGTG GTGACTACTG TTTTCTAAA AATGGGACAA TCTGTGTCCA	1380
65	CTCCCCTTTC TCTGACTCTG GTTCTGTCGC TTGGTAATTT TGTTTGTGTTA CGTTTGTGTT	1440

	TGTGAGTCGT CTATGTTGTC TGTTACTATC TTGTTTTGT TTGTGGTTA CGGTTCTGT	1500
5	GTGTGTCTTG TGTGTCTCTT TGTGTTCAGA CTTGGACTGA TGACTGACGA CTGTTTTAA	1560
	GTTATGCCTT CTAAAATAAG CCTAAAAATC CTGTCAGATC CCTATGCTGA CCACCTCCTT	1620
	TCAGATCAAC AGCTGCCCTT ACGTATCGAT GGATCCCTCG ACTAACTAAT AGCCCATTCT	1680
10	CCAAGGTCGA GCGGGATCAA TTCCGCCCCC CCCCTAACGT TACTGGCCGA AGCCGCTTGG	1740
	AATAAGGCCG GTGTGCGTTT GTCTATATGT TATTTCCAC CATATTGCCG TCTTTGGCA	1800
15	ATGTGAGGGC CGGGAAACCT GGCCCTGTCT TCTTGACGAG CATTCTAGG GGTCTTCCC	1860
	CTCTGCCAA AGGAATGCAA GGTCTGTTGA ATGTCGTGAA GGAAGCAGTT CCTCTGGAAG	1920
	CTTCTTGAAG ACAAAACAACG TCTGTAGCGA CCCTTGAG GCAGCGAAC CCCCCACCTG	1980
20	GCGACAGGTG CCTCTGCGGC CAAAGCCAC GTGTATAAGA TACACCTGCA AAGGCGGCAC	2040
	AACCCAGTG CCACGTTGTG AGTTGGATAG TTGTGGAAAG AGTCAAATGG CTCTCCTCAA	2100
25	GCGTATTCAA CAAGGGGCTG AAGGATGCC AGAAGGTACC CCATTGTATG GGATCTGATC	2160
	TGGGGCCTCG GTGCACATGC TTTACATGTG TTTAGTCGAG GTTAAAAAAA CGTCTAGGCC	2220
	CCCCGAACCA CGGGGACGTG GTTTCCCTT GAAAAACACG ATAATAATCA TGGCTACAGG	2280
30	CTCCCCGACG TCCCTGCTCC TGGCTTTGG CCTGCTCTGC CTGCCCTGGC TTCAAGAGGG	2340
	CAGTGCCTTC CCAACCATTG CCTTATCCAG GCTTTTGAC AACGCTATGC TCCGCGCCCA	2400
35	TCGTCTGCAC CAGCTGGCCT TTGACACCTA CCAGGAGTTT GAAGAAGCCT ATATCCAAA	2460
	GGAACAGAACAG TATTCAATTCC TGCAGAACCC CCAGACCTCC CTCTGTTCT CAGAGTCTAT	2520
	TCCGACACCC TCCAACAGGG AGGAAACACA ACAGAAATCC AACCTAGAGC TGCTCCGCAT	2580
40	CTCCCTGCTG CTCATCCAGT CGTGGCTGGA GCCCGTGCAG TTCCTCAGGA GTGTCTTCGC	2640
	CAACAGCCTG GTGTACGGCG CCTCTGACAG CAACGTCTAT GACCTCTAA AGGACCTAGA	2700
45	GGAAGGCATC CAAACGCTGA TGGGGAGGCT GGAAGATGGC AGCCCCCGGA CTGGGCAGAT	2760
	CTTCAAGCAG ACCTACAGCA AGTTGACAC AAACTCACAC AACGATGACG CACTACTCAA	2820
	GAACTACGGG CTGCTCTACT GCTTCAGGAA GGACATGGAC AAGGTCGAGA CATTCTGCG	2880
50	CATCGTGCAG TGCCGCTCTG TGGAGGGCAG CTGTGGCTTC TAGCTGCCCG GGTGGCATCC	2940
	TGTGACCCCT CCCCAGTGCC TCTCCTGGCC CTGGAAGTTG CCACTCCAGT GCCCACCCAGC	3000
55	CTTGTCTCAA TGTGTGTCAG TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA	3060
	GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC CCAGGCTCCC	3120
	CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC	3180
60	TAACTCCGCC CATCCCGCCC CTAACTCCGC CCAGTTCCGC CCATTCTCCG CCCCATGGCT	3240
	GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCCTGTAG CTATTCCAGA	3300
65	AGTAGTGAGG AGGTTTTTT GGAGGCCTAG GCTTTGCAA AAAGCTTCAC GCTGCCGCAA	3360
	GCACTCAGGG CGCAAGGGCT GCTAAAGGAA GCGGAACACG TAGAAAGCCA GTCCCGAGAA	3420

	ACGGTGCTGA CCCCGGATGA ATGTCAGCTA CTGGGCTATC TGGACAAGGG AAAACGCAAG	3480
5	CGCAAAGAGA AAGCAGGTAG CTTGCAGTGG GCTTACATGG CGATAGCTAG ACTGGCGGT	3540
	TTTATGGACA GCAAGCGAAC CGGAATTGCC AGCTGGGGCG CCCTCTGGTA AGGTTGGAA	3600
	GCCCTGCAAA GTAAAATGCCA TGGCTTCTT GCCGCCAAGG ATCTGATGGC GCAGGGGATC	3660
10	AAGATCTGAT CAAGAGACAG GATGAGGATC GTTTCGCATG ATTGAACAAG ATGGATTGCA	3720
	CGCAGGTTCT CCGGCCGCTT GGGTGGAGAG GCTATTGCCG TATGACTGGG CACAACAGAC	3780
	AATCGGCTGC TCTGATGCCG CCGTGTCCG GCTGTCAGCG CAGGGGCCCG CGGTTCTTT	3840
15	TGTCAAGACC GACCTGTCCG GTGCCCTGAA TGAACACTGCAG GACGAGGCAG CGCGGCTATC	3900
	GTGGCTGGCC ACGACGGGCG TTCCCTGCGC AGCTGTGCTC GACGTTGTCA CTGAAGCGGG	3960
20	AAGGGACTGG CTGCTATTGG GCGAAGTGCC GGGGCAGGAT CTCCCTGTCAT CTCACCTTGC	4020
	TCCTGCCGAG AAAGTATCCA TCATGGCTGA TGCAATGCCG CGGCTGCATA CGCTTGATCC	4080
	GGCTACCTGC CCATTGACCC ACCAAGCGAA ACATCGCATC GAGCGAGCAC GTACTCGGAT	4140
25	GGAAGCCGGT CTTGTCGATC AGGATGATCT GGACGAAGAG CATCAGGGGC TCGGCCAGC	4200
	CGAACACTGTC GCCAGGCTCA AGGCGCGCAT GCCCGACGGC GAGGATCTCG TCGTGACCCA	4260
30	TGGCGATGCC TGCTTGCCGA ATATCATGGT GGAAAATGGC CGCTTTCTG GATTGATCGA	4320
	CTGTGGCCGG CTGGGTGTGG CGGACCGCTA TCAGGACATA SCGTTGGCTA CCCGTGATAT	4380
	TGCTGAAGAG CTTGGCGGCG AATGGGCTGA CCGCTTCCTC GTGCTTTACG GTATGCCGC	4440
35	TCCCGATTG CAGCGCATCG CCTTCTATCG CCTTCTTGAC GAGTTCTTCT GAGCGGGACT	4500
	CTGGGGTTCG AAATGACCGA CCAAGCGACG CCCAACCTCC AGAAAAAAGGG GGGAAATGAAA	4560
40	GACCCCACCT GTAGGTTTGG CAAGCTAGCT TAAGTAACGC CATTGCAA GGCATGGAAA	4620
	AATACATAAC TGAGAAATAGA GAAGTTCAGA TCAAGGTCAG GAACAGATGG AACAGCTGAA	4680
	TATGGGCCAA ACAGGATATC TGTGGTAAGC AGTTCTGCC CGGGCTCAGG GCCAAGAAC	4740
45	GATGGAACAG CTGAATATGG GCCAAACAGG ATATCTGTGG TAAGCAGTTC CTGCCCCGGC	4800
	TCAGGGCCAA GAACAGATGG TCCCCAGATG CGGTCCAGCC CTCAGCAGTT TCTAGAGAAC	4860
	CATCAGATGT TTCCAGGGTG CCCCAAGGAC CTGAAATGAC CCTGTCCTT ATTTGAAC	4920
50	ACCAATCAGT TCGCTTCTCG CCTCTGTTG CGCGCTCTG CTCCCCGAGC TCAATAAAAG	4980
	AGCCCACAAAC CCCTCACTCG GGGCGCCAGT AATCTGCTGC TTGCAAACAA AAAAACCAACC	5040
55	GCTACCAGCG GTGGTTTGTG TGCCGGATCA AGAGCTACCA ACTCTTTTC CGAAGGTAAC	5100
	TGGCTTCAGC AGAGCGCAGA TACCAAATAC TGTCTTCTA GTGTAGCCGT AGTTAGGCCA	5160
60	CCACTTCAAG AACTCTGTAG CACCGCCTAC ATACCTCGCT CTGCTAATCC TGTTACCAGT	5220
	GGCTGCTGCC AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC GATAGTTACC	5280
	GGATAAGGCG CAGCGGTGG GCTGAACGGG GGGTTCCGTGC ACACAGCCCA GCTTGGAGCG	5340
65	AACGACCTAC ACCGAACCTGA GATACCTACA GCGTGAGCAT TGAGAAAGCG CCACGCTTCC	5400

CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCAGGAGG GTCGGAACAG GAGAGCGCAC 5460  
 GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTCGGGT TTGCCACCT 5520  
 5 CTGACTTGAG CGTCGATTTT TGTGATGCTC GTCAGGGGG CGGAGCCTAT GGAAAAACGC 5580  
 CAGCAACGCC GAGA 5594

## (2) INFORMATION FOR SEQ ID NO:30:

- 10 (i) SEQUENCE CHARACTERISTICS:  
     (A) LENGTH: 6561 base pairs  
     (B) TYPE: nucleic acid  
     (C) STRANDEDNESS: single  
 15     (D) TOPOLOGY: linear  
       (ii) MOLECULE TYPE: DNA (genomic)

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:  
 GATCCCCGGG TCGACCCGGG TCGACCCGTG GGAATGTGTG TCAGTTAGGG TGTGGAAAGT 60

25 CCCCAGGCTC CCCAGCAGGC AGAAGTATGC AAAGCATGCA TCTCAATTAG TCAGCAACCA 120  
 GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT 180  
 30 AGTCAGCAAC CATAGTCCCG CCCCTAACTC CGCCCATCCC GCCCTAACT CCGCCAGTT 240  
 CCGCCCATTC TCCGCCCAT GGCTGACTAA TTTTTTTAT TTATGCAGAG GCGGAGGCCG 300  
 35 CCTCGGCCTC TGAGCTATTG CAGAAGTAGT GAGGAGGCTT TTTTGGAGGC CTAGGCTTT 360  
 GCAAAAAGCT TCACGCTGCC GCAAGCACTC AGGGCGCAAG GGCTGCTAAA GGAAGCGGAA 420  
 CACGTAGAAA GCCAGTCCGC AGAAACGGTG CTGACCCCGG ATGAATGTCA GCTACTGGGC 480  
 40 TATCTGGACA AGGGAAAACG CAAGCGAAA GAGAAAGCAG GTAGCTTGCA GTGGGCTTAC 540  
 ATGGCGATAG CTAGACTGGG CGGTTTTATG GACAGCAAGC GAACCGGAAT TGCCAGCTGG 600  
 45 GGCGCCCTCT GGTAAAGGTTG GGAAGCCCTG CAAAGTAAAC TGGATGGCTT TCTTGCCGCC 660  
 AAGGATCTGA TGGCGCAGGG GATCAAGATC TGATCAAGAG ACAGGATGAG GATCGTTTCG 720  
 CATGATTGAA CAAGATGGAT TGCACGCAGG TTCTCCGGCC GCTTGGGTGG AGAGGCTATT 780  
 50 CGGCTATGAC TGGGCACAAC AGACAATCGG CTGCTCTGAT GCCGCGTGT TCCGGCTGTC 840  
 AGCGCAGGGG CGCCCGGTTTC TTTTGTCAG GACCGACCTG TCCGGTGCCTC TGAATGAAC 900  
 55 GCAGGACGAG GCAGCGCGGC TATCGTGGCT GGCCACGACG GGCGTTCCCTT GCGCAGCTGT 960  
 GCTCGACGTT GTCACTGAAG CGGGAAAGGGA CTGGCTGCTA TTGGGCGAAG TGCCGGGGCA 1020  
 GGATCTCCTG TCATCTCACC TTGCTCCTGC CGAGAAAGTA TCCATCATGG CTGATGCAAT 1080  
 60 GCGGCGGCTG CATAACGCTTG ATCCGGCTAC CTGCCCATTC GACCACCAAG CGAACACATCG 1140  
 CATCGAGCGA GCACGTACTC GGATGGAAGC CGGTCTTGTC GATCAGGATG ATCTGGACGA 1200  
 AGAGCATCAG GGGCTCGCGC CAGCCGAACG GTTCGCCAGG CTCAAGGCCGC GCATGCCCGA 1260  
 65 CGGCGAGGAT CTCGTCGTGA CCCATGGCGA TGCCTGCTTG CCGAATATCA TGGTGGAAAA 1320

	TGGCCGCTTT TCTGGATTCA TCGACTGTGG CCGGCTGGGT GTGGCGGACC GCTATCAGGA	1380
5	CATAGCGTTG GCTACCCGTG ATATTGCTGA AGAGCTTGGC GGCGAATGGG CTGACCGCTT	1440
	CCTCGTGCTT TACGGTATCG CCGCTCCCGA TTCGCAGCGC ATCGCCTTCT ATCGCCTTCT	1500
	TGACGAGTTC TTCTGAGCGG GACTCTGGGG TTCGAAATGA CCGACCAAGC GACGCCAAC	1560
10	CTGCCATCAC GAGATTCGA TTCCACCGCC GCCTTCTATG AAAGGTTGGG CTTCGGAATC GTTTCCGGG ACGCCGGCTG GATGATCCTC CAGCGCGGGG ATCTCATGCT GGAGTTCTTC	1620 1680
	GCCCACCCCG GAATTCTGAA TCTGCTGCTT GCAAACAAAA AAACCACCGC TACCAGCGGT	1740
15	GGTTTGTGTTG CCGGATCAAG AGCTACCAAC TCTTTTCCG AAGGTAAC TG GCTTCAGCAG AGCGCAGATA CCAAATACTG TCCTTCTAGT TAGGCCGTAG TAGGCCACC ACTTCAAGAA	1800 1860
20	CTCTGTAGCA CCCGCTACAT ACCTCGCTCT GCTAATCCTG TTACCAAGTG CTGCTGCCAG TGGCGATAAG TCGTGTCTTA CGGGGTTGGA CTCAAGACGA TAGTTACCGG ATAAGGCAGCA	1920 1980
	GCGGTGGGC TGAACGGGGG GTTCGTGCAC ACAGCCCAGC TTGGAGCGAA CGACCTACAC	2040
25	CGAACTGAGA TACCTACAGC GTGAGCATTG AGAAAGCGCC ACGCTTCCCG AAGGGAGAAA GGCGGACAGG TATCCGGTAA GCGGCAGGGT CGGAACAGGA GAGCGCACGA GGGAGCTTCC	2100 2160
30	AGGGGGAAAC GCCTGGTATC TTTATAGTCC TGTGGGTTT CGCCACCTCT GACTTGAGCG TCGATTGTTG TGATGCTCGT CAGGGGGGCG GAGCCTATGG AAAAACGCCA GCAACGCCGA	2220 2280
	GATGCGCCGC CTCGAGTACA CCTCGTCAT GCTGAGACCC TCAAGCCTCA CTAAAAGGGT	2340
35	CCCTGCCTAG TTCTGTTAC TAATCTGCCT TATTCTGTTT TTGTTCCCAT GTTAAAGATA GAGTAAATGC AGTATTCTCC ACATAGAGAT ATAGACTTCT GAAATTCTAA GATTAGAATT	2400 2460
40	ATTTACAAGA AGAAGTGGGG AATGAAGAAAT AAAAATTAC TGGCCTCTTG TGAGAACATG AACTTTCACC TCGGAGCCCA CCCCCCTCCA TCTGGAAAAC ATACTTGAGA AAAACATTTC	2520 2580
	CTGGAACAAAC CACAGAATGT TTCAACAGGC CAGATGTATT GCCAAACACA GGATATGACT	2640
45	CTTTGGTTGA GTAAATTGTG GGTTGTTAAA CTTCCCTAT TCCCTCCCCA TTCCCCCTCC CAGTTGTGG TTTTTCCCTT TAAAAGCTTG TGAAAAAATTG GAGTCGTCGT CGAGACTCCT	2700 2760
	CTACCCGTG CAAAGGTGTA TGAGTTCGA CCCCAGAGCT CTGTGTGCTT TCTGTTGCTG	2820
50	CTTTATTTCG ACCCCAGAGC TCTGGTCTGT GTGCTTCAT GTCGCTGCTT TATTAATCT TACCTTCTAC ATTTTATGTA TGGTCTCAGT GTCTCTTGG GTACGCGGCT GTCCCGGGAC	2880 2940
55	TTGAGTGTCT GAGTGAGGGT CTTCCCTCGA GGGCTTTCA TTTGGTACAT GGGCCGGGAA TTCGAGAAC TTTCAATTGG TGCAATTGCC GGGAAATCGA AAATCTTCA TTTGGTGCAT	3000 3060
	TGGCCGGAA ACAGCGCGAC CACCCAGAGG TCCTAGACCC ACTTAGAGGT AAGATTCTT	3120
60	GTTCTGTTT GGTCTGATGT CTGTGTCTG ATGTCTGTGT TCTGTTCTA AGTCTGGTGC GATCGCAGTT TCAGTTTGC GGACGCTCAG TGAGACCGCG CTCCGAGAGG GAGTGCAGGG	3180 3240
65	TGGATAAGGA TAGACGTGTC CAGGTGTCCA CCGTCCGTTC GCCCTGGGAG ACGTCCCAGG	3300

	AGGAACAGGG GAGGATCAGG GACGCCTGGT GGACCCCTT GAAGGCCAAG AGACCATTG	3360
	GGGTTGCGAG ATCGTGGTT CGAGTCCCAC CTCGTGCCA GTTGCAGAT CGTGGGTCG	3420
5	AGTCCCACCT CGTGTTTGT TGCGAGATCG TGGGTTGAG TCCCACCTCG CGTCTGGTCA	3480
	CGGGATCGTG GGTCGAGTC CCACCTCGTG TTTGTTGCG AGATCGTGGG TTCGAGTCCC	3540
10	ACCTCGCGTC TGGTCACGGG ATCGTGGGTT CGAGTCCCAC CTCGTGCAGA GGGTCTCAAT	3600
	TGGCCGGCCT TAGAGAGGCC ATCTGATTCT TCTGTTTCT CTTTTGTCT TAGTCTCGTG	3660
	TCCGCTCTTG TTGTGACTAC TGTTTTCTA AAAATGGGAC AATCTGTGTC CACTCCCCTT	3720
15	TCTCTGACTC TGGTTCTGTC GCTTGGTAAT TTTGTTGTT TACGTTTGT TTTGTGAGTC	3780
	GTCTATGTTG TCTGTTACTA TCTTGTTTT GTTTGTGGTT TACGGTTTCT GTGTGTGTCT	3840
	TGTGTGTCTC TTTGTGTTCA GACTTGGACT GATGACTGAC GACTGTTTT AAGTTATGCC	3900
20	TTCTAAAATA AGCCTAAAAAA TCCTGTCAAGA TCCCTATGCT GACCACTTCC TTTCAGATCA	3960
	ACAGCTGCCCG TGCCCTCCCAC TCCAACCTCCA GAGAGCAGCC AGCGGGTCAC AGTGGTCCCG	4020
25	CCCATGAACC TGGAGCCTAG GGAAAAATGA GCTCGGAAAT CCGGAGCAAA TGAGGAGTGG	4080
	TCCCTGAGAA GTCACTGGCC TAAATGTTGT GGCTGCTGAA GCAGAGAAG AGGAGGCTGT	4140
	TCGAGTAGCC GGCCAAGAGC GCCGCGGGTT CCCAGGCAGC TTCTCATTCC CCTGTCCCTC	4200
30	CCATCCCGTC TCTTGTAAAC AGAAAAACTG CTTTCACTTT GAGATATGAG TGGCCCGATA	4260
	CAGCCAGCTG TGAGAGCTGT ACTCCCTTCC CTGCCAACG TGTTTCTCT TCTCAGGCAGA	4320
35	CCCCCTCCCTG AGCTGCTGGC AGTGAGCTG TTCTAAGCTC CAGTGAGGGA GGCATCCGCC	4380
	CACTTGGGGC TTCTGTCCAA GGTAAGGAGC ACCTGTGAGT CTAACGCCA GGCTCTGATG	4440
	GGGGTCTCGT CTCTGTGGGA CTAGAAAGTG TCCCAACAAT CTGACCAAGG TAACAGGAAG	4500
40	TTAAGACAAA GACAGAGACC AAAGTCAGAA TCAGAGCTGT GCTGTGAGAC AAAAGATAA	4560
	AAAAAAATAAA ATGCTGGCCA CAAAAGTCAG GAAAACAGA AAACCTAGAT AGTACCTGGC	4620
45	AACAAAAGAA AGCTTTGGC TAAAGATCAA CGTGTATACT GTAAAGAAAA TGAGCACTGG	4680
	GTGAGAGACT GCCCAACAA AAAGAAGAGG AGCCCCCTC ATGACCAAC CCTCACCTG	4740
	TTCGTGGCTA AAAGTAAAGA GATAACAAA GGGGTGCTAA CACAGAAGCT GAGTCCTTAA	4800
50	AAGAGTCCGG TGGCTACCT GTTGAAGCAG CTAAAAAAGA GACTGTGTTT CATACTCCTC	4860
	CACTGACCAG TGCAAAACAA GCTAAAAGT TCCTGGCAC TGCAGGGCTTT TGCAGATTGT	4920
55	GGATTCCAGG TTTGCTGAG TTAAAGAGAT AAACAGCCCT TCGTATAGAA AAATAAAAAA	4980
	CAACCTTGGGA TGCTCTTGGGA TGCTATTGAG ACTGCCCTAA TGTTGTCCCC AGCTATGGGA	5040
	CTCCTAGATG TGACTGAGAA CAAAGGTATT GCCAAAGAAG TTCTTACTCA GAGATTGGGA	5100
60	CCCTGAAAAA GACCTGTGGC ATACTTGTAA GAAATTAGAC CTGGTGGCTG TAAGATGGCC	5160
	TGCTTGTCTG CACATAGTGG CTTCTGGTCA AGGACGCAGA TAAATTGACT CTGAGACAAA	5220
65	ACTTGGCACA TGTCTAGAA AGTGTGGTTC AGCCCCCATG ACCGATGGCT GACTAACGCT	5280
	CTTGAAAACA TTATCCAAT GTTCCCTGAGA CCGATGGACA CATTGTCAGA GCTTTTTTG	5340

	ACTGAACGAG	TGACCTTCGC	TCCCCCTGCT	ATCCTCGATC	TCACTACTGC	CTGAGACTTC	5400
5	ACCTACTCAT	CATTGTGCTG	ACATTCTGGC	AGAAGAAA	ACT CATACTCGAA	ATGATCTGAA	5460
	GGATCAGATC	AGCCTTGGCC	TGAGAGTTTG	AGCTGGTACA	CGGATGGCAG	TAGCCTGGAG	5520
	GTAAAGGGTA	AGCGGAAGGC	GGGGACAGCA	GTGCA GTGGT	GGACAGAAAG	CAAGTGATCT	5580
10	AGGCCAGCAG	CCTCCCTAAA	GGGACTTCAG	CCCACAAAGC	CAAAC TTGTG	GCTTTAATAC	5640
	AAGCTCTGTA	AATGGTAAAAA	AAAAAAAAGT	CTACACGGAC	AGCAGGTATG	CTCTTGCCAC	5700
	TGTACAGAGC	AATATACAGA	CAAAGAGAAC	TGTTGACATC	TGCAGAGAAA	GACCTAAGAT	5760
15	GCTGTGGCTA	AAAGAAATCA	GATGGCAAAT	CTAACCGCCC	AGGCATCCTA	AAGAGCAATG	5820
	ATCCTGACAG	TCTGAAGACT	ATCAAGTTAT	AGACAAATT	AGACTGGTAA	AAAAAAACCT	5880
20	GTATAAAATA	GTAAAAAACTG	AAAAAAGAAA	ACTAGTCCTC	TCATGAGAAG	ACAGACCTGA	5940
	CATCTACTGA	AAAATAGACT	TTACTGGAAA	AAATATGTGT	ATGAATACCT	TCTAGTTTT	6000
	GTGAACGTT	TCAAGATGGA	TAAAAGCTTT	TCCTTGTA	ACGAGACTGA	TCAGATAGTC	6060
25	ATCAAGAAGA	TTGTTAAAGA	AAATTTCCA	AGGTTCGGAG	TGCCAAAAGC	AATAGTGTCA	6120
	GATAATGGTC	CTGCCCTTGT	TGCCCAAGGT	AGTCAGGGTG	TGGCCAAGTA	TTTAGAGGTC	6180
30	AAATGAAAAT	TCCATTGTGT	GTACAGACCT	CAGAGCTCAG	GAAAGATAAA	AAAGAATAAA	6240
	AAAAACTCTA	AACAGACCTT	GACAAAATT	ATCCTAGAGA	CTGGCACAGA	CTTACTTGGT	6300
	ACTCCTTCCC	CTTGCCCTAT	TTAGAACTGA	GAATACTCCC	TCTTGATTG	GTTTTACTCT	6360
35	TTTTAAGATC	CTTTATGGGG	CTCCTATGCC	ATCACTGTCT	TAAATGATGT	GTTTAAACCT	6420
	ATGTTGTTAT	AATAATGATC	TATATGTTAA	GTTAAAAGGC	TTGCAGGTGG	TGCAGAAAGA	6480
40	AGTCTGGTCA	CAACTGGCTA	CAGTGAACAA	GCTGGGTACC	CCAAGGACAT	CTTACCAGTT	6540
	CCAGCCAGAG	ATCTGATCTA	C				6561

## (2) INFORMATION FOR SEQ ID NO:31:

45 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 55 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: DNA (genomic)

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GACTAACCTT GATTCCACTG GAGCCGTATT ACCGCCATGC ATTAGTTATT AATAG 55

## 60 (2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 47 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GACTAACCTT GATTCCACTG GAGTAATTGC GGCTAGCGGA TCTGACG

47

15 (2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 66 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: DNA (genomic)

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GACTAACCTT GATTCCACTG GAGACACTTG ACCTCTACCG CGCCAGTCCT CCGATTGACT  
GAGTCG

60

66

30 (2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 48 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: DNA (genomic)

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GACTAACCTT GATTCCACTG GAGGGATCCG CGCCCATGAT TATTATCG

48

45 (2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 55 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: DNA (genomic)

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GACTAACCTT GATTCCAGCA ATGTCATGGC TACAGGCTCC CGGACGTCCC TGCTC

55

60 (2) INFORMATION FOR SEQ ID NO:36:

65 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 48 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GACTAACCTT GATTCCAGCA ATGTTAGGAC AAGGCTGGTG GGCAGTGG

48

(2) INFORMATION FOR SEQ ID NO:37:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 49 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: DNA (genomic)

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GACTAACCTT GATTCCACTG GAGGGTCGAC CCTGTGGAAT GTGTGTCAG

49

30 (2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 48 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: DNA (genomic)

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

45 GACTAACCTT GATTCCACTG GAGAATCTCG TGATGGCAGG TTGGGCGT

48

(2) INFORMATION FOR SEQ ID NO:39:

50 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 54 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: DNA (genomic)

60 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GACTAACCTT GATTCCACTG AAGAGATTTT ATTTAGTCTC CAGAAAAAGG GGGG

54

(2) INFORMATION FOR SEQ ID NO:40:

65 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

5 (iii) MOLECULE TYPE: DNA (genomic)

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

GAATTAACCTT GATTCCACTG AAGCCCCCAA ATGAAAGACC CCCGCTGACG

50

15 (2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:

  - (A) LENGTH: 49 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

30 GACTAACCTT GATTCCACTG GAGCCGGGAC GGAATTCGTA ATCTGCTGC

49

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:

  - (A) LENGTH: 47 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: DNA (genomic)

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GAATACCTT GATTCCACTG GAGTTCTCGA GGCGGGCGCAT CTCGGCG

47

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:

  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CGCTCTAGAA CTAGTGGATC

20

65

5                   (i) SEQUENCE CHARACTERISTICS:  
                     (A) LENGTH: 21 base pairs  
                     (B) TYPE: nucleic acid  
                     (C) STRANDEDNESS: single  
                     (D) TOPOLOGY: linear

10                  (ii) MOLECULE TYPE: DNA (genomic)

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

15   GTAATACGAC TCACTATAGG G

21

(2) INFORMATION FOR SEQ ID NO:45:

20                  (i) SEQUENCE CHARACTERISTICS:  
                     (A) LENGTH: 43 base pairs  
                     (B) TYPE: nucleic acid  
                     (C) STRANDEDNESS: single  
                     (D) TOPOLOGY: linear

25                  (ii) MOLECULE TYPE: DNA (genomic)

30                  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CGATCCACTG GAGCTCGGAG CCCACCCCT CCCATCTAGA GGT

43

(2) INFORMATION FOR SEQ ID NO:46:

35                  (i) SEQUENCE CHARACTERISTICS:  
                     (A) LENGTH: 43 base pairs  
                     (B) TYPE: nucleic acid  
                     (C) STRANDEDNESS: single  
                     (D) TOPOLOGY: linear

40                  (ii) MOLECULE TYPE: DNA (genomic)

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

50   CGTCCTCTG GAGAGCACAG GGTAGAGGGAG TCTCGACGGT CAG

43

(2) INFORMATION FOR SEQ ID NO:47:

55                  (i) SEQUENCE CHARACTERISTICS:  
                     (A) LENGTH: 43 base pairs  
                     (B) TYPE: nucleic acid  
                     (C) STRANDEDNESS: single  
                     (D) TOPOLOGY: linear

60                  (ii) MOLECULE TYPE: DNA (genomic)

65                  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CGCAACCCTG GAGACCTCTA GATGGGAGGG GGTGGGCTCC GAG

43

## (2) INFORMATION FOR SEQ ID NO:48:

5           (i) SEQUENCE CHARACTERISTICS:  
              (A) LENGTH: 43 base pairs  
              (B) TYPE: nucleic acid  
              (C) STRANDEDNESS: single  
              (D) TOPOLOGY: linear

10          (ii) MOLECULE TYPE: DNA (genomic)

15          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GCAGGACCTG GAGCTGACCG TCGAGACTCC TCTACCCTGT GCT

43

## (2) INFORMATION FOR SEQ ID NO:49:

20           (i) SEQUENCE CHARACTERISTICS:  
              (A) LENGTH: 20 base pairs  
              (B) TYPE: nucleic acid  
              (C) STRANDEDNESS: single  
              (D) TOPOLOGY: linear

25          (iii) MOLECULE TYPE: DNA (genomic)

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CGCTCTAGAA CTAGTGGATC

20

## (2) INFORMATION FOR SEQ ID NO:50:

35           (i) SEQUENCE CHARACTERISTICS:  
              (A) LENGTH: 21 base pairs  
              (B) TYPE: nucleic acid  
              (C) STRANDEDNESS: single  
              (D) TOPOLOGY: linear

40          (iii) MOLECULE TYPE: DNA (genomic)

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

50          GTAATACGAC TCACTATAGG G

21

## (2) INFORMATION FOR SEQ ID NO:51:

55           (i) SEQUENCE CHARACTERISTICS:  
              (A) LENGTH: 19 base pairs  
              (B) TYPE: nucleic acid  
              (C) STRANDEDNESS: single  
              (D) TOPOLOGY: linear

60          (ii) MOLECULE TYPE: DNA (genomic)

65

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

TACGTATCGA TGGATCCGA

19

(2) INFORMATION FOR SEQ ID NO:52:

- 5       (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 19 base pairs  
          (B) TYPE: nucleic acid  
          (C) STRANDEDNESS: single  
          (D) TOPOLOGY: linear

10      (ii) MOLECULE TYPE: DNA (genomic)

15      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:  
GGATCCATCG ATACGTAAG

19

(2) INFORMATION FOR SEQ ID NO:53:

- 20       (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 38 base pairs  
          (B) TYPE: nucleic acid  
          (C) STRANDEDNESS: single  
          (D) TOPOLOGY: linear

25      (ii) MOLECULE TYPE: DNA (genomic)

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

35      GGCCGCTAAC TAATAGCCCCA TTCTCCAAGG TACGTAGC

38

(2) INFORMATION FOR SEQ ID NO:54:

- 40       (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 38 base pairs  
          (B) TYPE: nucleic acid  
          (C) STRANDEDNESS: single  
          (D) TOPOLOGY: linear

45      (ii) MOLECULE TYPE: DNA (genomic)

50      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:  
TACGTACCTT GGAGAAATGGG CTATTAGTTA GCGGCCGC

38

(2) INFORMATION FOR SEQ ID NO:55:

- 55       (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 55 base pairs  
          (B) TYPE: nucleic acid  
          (C) STRANDEDNESS: single  
          (D) TOPOLOGY: linear

60      (ii) MOLECULE TYPE: DNA (genomic)

65

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:  
GACTAACCTT GATTCCACTG GAGTTTCTC TATTCTTCAT TCCCCACTTC TTCTT

55

## (2) INFORMATION FOR SEQ ID NO:56:

5

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 60 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: DNA (genomic)

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

20

GACTAACCTT GATTCCACTG GAGAATCTGG ACCAATTCTA TATAAGCCTG TGAAAAATT

60

## (2) INFORMATION FOR SEQ ID NO:57:

25

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 46 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: DNA (genomic)

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GACTAACCTT GATTCCACTG GAGAAGAAGA AGTGGGAAT GAAGAA

46

40

## (2) INFORMATION FOR SEQ ID NO:58:

45

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 51 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GACTAACCTT GATTCCACTG GAGATCTCTA GATGGGAGGG GGTCTGGGCT C

51

55

## (2) INFORMATION FOR SEQ ID NO:59:

60

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 47 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

65

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

5 GACTAACCTT GATTCCACTG GAGCTCGGAG CCCACCCCT CCCATCT

47

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

20 GACTAACCTT GATTCCACTG GAGGGAGGCC CTTATCTCAA AAATGTT

47

(2) INFORMATION FOR SEQ ID NO:61:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: DNA (genomic)

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GACTAACCTT GATTCCACTG GAGTCATAAGA ACATTTTG AATAAGGGCC T

51

40 (2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 44 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: DNA (genomic)

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

55 GACTAACCTT GATTCCACTG GAGTCACAGG CTTATATAGT GAAA

44

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

60 65 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

5 GACTAACCTT GATTCCCTGG AGACTGCACT GCTGTCCCCG CCTTCG

46

(2) INFORMATION FOR SEQ ID NO:64:

10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 53 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: DNA (genomic)

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GAGTAACCTT GATTCCCTGG AGATTTCTCA GACCCGGGTC GACCCTGTGG AAT

53

25 (2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 44 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: DNA (genomic)

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GACTAACCTT GATTCCCTGG AGCTCGAGGC GGCGCATCTC GGCG

44

40 (2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: DNA (genomic)

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GACTAACCTT GATTCCCTGA AGACCTGCGT CATGCTGAGA CCCTCAA

47

55 (2) INFORMATION FOR SEQ ID NO:67:

60 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

65 (ii) MOLECULE TYPE: DNA (genomic)

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

GACTAACCTT GATTCCCTGA AGCGGCCAAT GCACCAAATG AAAGATTTTC

50

(2) INFORMATION FOR SEQ ID NO:68:

10 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 50 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: DNA (genomic)

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

CGCATCTTTT AATTAACGG AGARAATTAA TYACAGGCTT ATATAGKAAA

50

We claim:

1. A method for assembling a gene or gene vector comprising the steps of:

5           a) designing at least 6 primers to produce at least three fragments in at least three separate polymerase chain reactions wherein each primer comprises at least one predetermined restriction endonuclease recognition site that recognizes a restriction endonuclease that cleaves at a distance from the recognition site, a sequence complementary to a template sequence for amplification, and bases positioned at the restriction endonuclease 10 cleavage site that are selected to be complementary to only one other overhanging created from enzymatic cleavage of the fragments;

15           b) combining the primers with template nucleic acid and performing a gene amplification reaction to produce multiple copies of an amplified template fragment incorporating the restriction endonuclease recognition site;

15           c) digesting the amplified template fragments with one or more restriction endonucleases that recognize the restriction endonuclease recognition site of the primers to create overhanging termini wherein each overhanging termini is complementary to only one other overhanging termini on another fragment; and

20           d) combining the amplified and digested template fragments in a ligation reaction to produce a directionally ordered gene, nucleic acid fragment or gene vector.

2. The method of claim 1 wherein the restriction endonuclease is at least one class IIS restriction endonuclease.

25           3. The method of claim 2 wherein the class IIS restriction endonuclease is selected from the group consisting of: *AlwI*, *Alw26I*, *BbsI*, *BbvI*, *BbvII*, *BpmI*, *BsmAI*, *BsmI*, *BsmBI*, *BspMI*, *BsrI*, *BsrDI*, *Eco57I*, *EarI*, *FokI*, *GsuI*, *HgaI*, *HphI*, *MboII*, *MnII*, *PleI*, *SapI*, *SfaNI*, *TaqII*, *Tth111II*.

30           4. The method of claim 1 wherein class II restriction endonuclease recognition sites, linkers, or adapters are not used to create the gene or gene vector.

5. The method of claim 1 wherein the product of the ligation reaction is introduced into prokaryotic or eukaryotic cells.

5 6. The method of claim 1 wherein at least one target nucleic acid sequence is chosen from the group consisting of : transcriptional regulatory sequences; genetic vectors; introns and/or exons; viral encapsidation sequences; integration signals intended for introducing nucleic acid molecules into other nucleic acid molecules; retrotransposon(s); VL30 elements; or multiple allelic forms of a sequence.

10

7. The method of claim 1 wherein the method is used to generate combinatorial libraries of a target sequence.

8. The method of claim 7 wherein the target sequence is part or all of a gene.

15

9. The method of claim 8 wherein the gene encodes a protein.

10. The method of claim 8 wherein the primers amplify allelic variants of part or all of a gene.

20

11. The method of claim 1 wherein the product of the ligation reaction is passed between eukaryotic cells using a virus particle, by cell fusion, or by transfection.

25

12. The method of claim 1 wherein the product of the ligation reaction is not introduced into prokaryotic cells.

13. The method of claim 1 further combining at least one screening or selection step to select the products of the ligation reaction.

30

14. The method of claim 1 wherein the product of the ligation reaction is mutated during passage in cells in order to generate genetic diversity.

15. The method of claim 14 wherein the product of the ligation reaction is mutated by homologous recombination during passage in cells.

16. The method of claim 1, wherein the method is used to isolate and identify regulatory  
5 sequences from a cell.

17. The method of claim 11, wherein cells containing the product of the ligation reaction are selected for enhanced biological activity.

10 18. The method of claim 17, wherein the cells containing the product of the ligation reaction are selected for tissue-specific, hormone-specific or developmental-specific gene expression.

15 19. The method of claim 1 wherein the product of the ligation reaction is a circularized gene vector.

20. A nucleic acid primer having a 5' and a 3' end to amplify a nucleic acid fragment for the ligation of at least two fragments comprising:

20 a restriction endonuclease recognition site that recognizes a restriction endonuclease,  
wherein the restriction endonuclease cleaves at a distance from the recognition site and  
creates overhanging termini;

a sequence complementary to a template sequence to be amplified to produce the  
nucleic acid fragment;

at least two nucleic acid bases positioned at the restriction endonuclease cleavage site  
25 and that form an overhanging terminus after cleavage by the restriction endonuclease,  
wherein the at least two nucleic acid bases are selected to be complementary to only one other  
overhanging terminus on another fragment of the ligation; and

an affinity handle on the 5' end of the primer.

30 21. The primer of claim 20 further comprising an anchor to provide stability to the  
restriction enzyme at the restriction enzyme recognition site.

22. A method for isolating and identifying promoters comprising the steps of:

a) obtaining a vector comprising at least a portion of a promoter region from a retrovirus transposon LTR and having two non-complementary overhanging termini;

b) designing at least two PCR primers to amplify at least one region of a

5 retro-transposon LTR from template nucleic acid to produce at least one nucleic acid fragment wherein each primer comprises at least one predetermined restriction endonuclease recognition site that recognizes a restriction endonuclease that cleaves at a distance from the recognition site, a sequence complementary to a template sequence from a retrovirus transposon, and bases positioned at the restriction endonuclease cleavage site that are selected

10 to be complementary to only one other overhanging terminus of the vector wherein the restriction endonuclease cleavage site is created from enzymatic cleavage of the fragments;

c) combining the primers with template nucleic acid and performing a gene amplification reaction to produce multiple copies of an amplified template fragment incorporating the restriction endonuclease recognition site;

15 d) digesting the amplified template fragments with one or more restriction endonuclease that recognize the restriction endonuclease recognition site of the primer to create overhanging termini; and

e) combining the amplified and digested template fragment in a ligation reaction with the vector to produce a gene vector with an intact LTR sequence.

20

23. The method of claim 22 wherein the template nucleic acid is DNA or RNA.

24. The method of claim 22 further comprising the step of sequencing the insert to identify the promoter sequence.

25

25. Promoter sequences of SEQ ID NOS:2-13 identified using the methods of claim 22.

26. The vector of SEQ ID NO:1.

30

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Fig 1A

A.

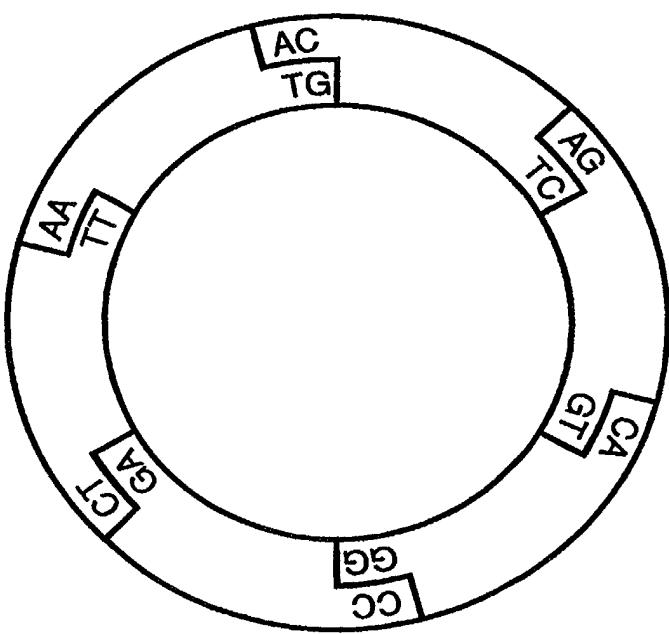
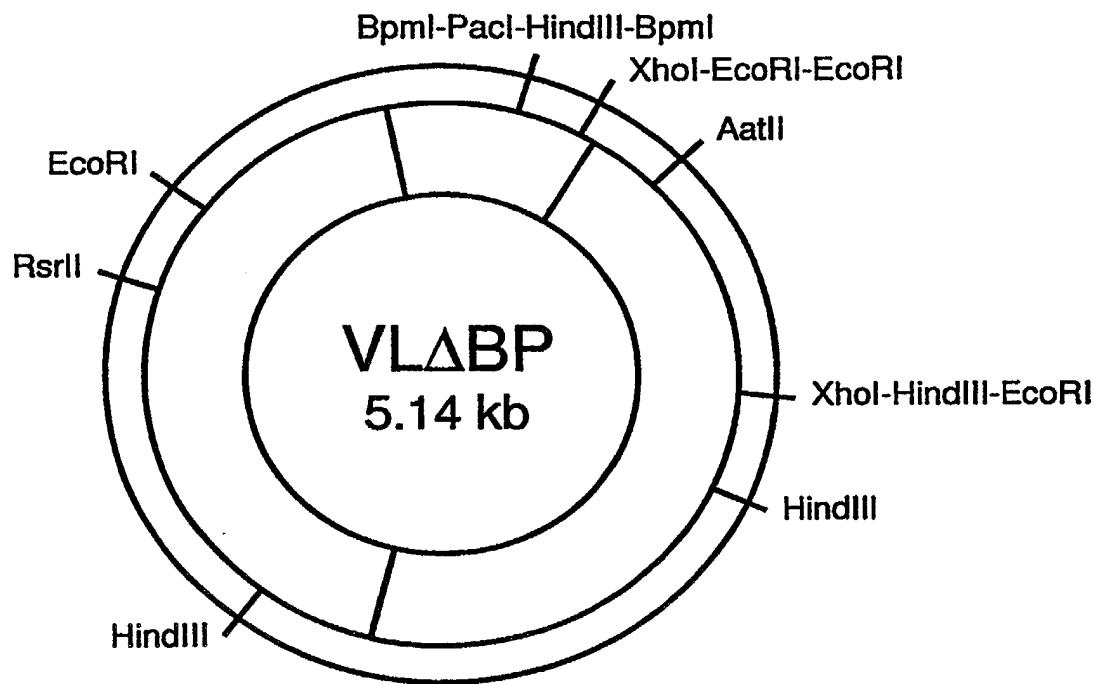


Fig 1B

B.



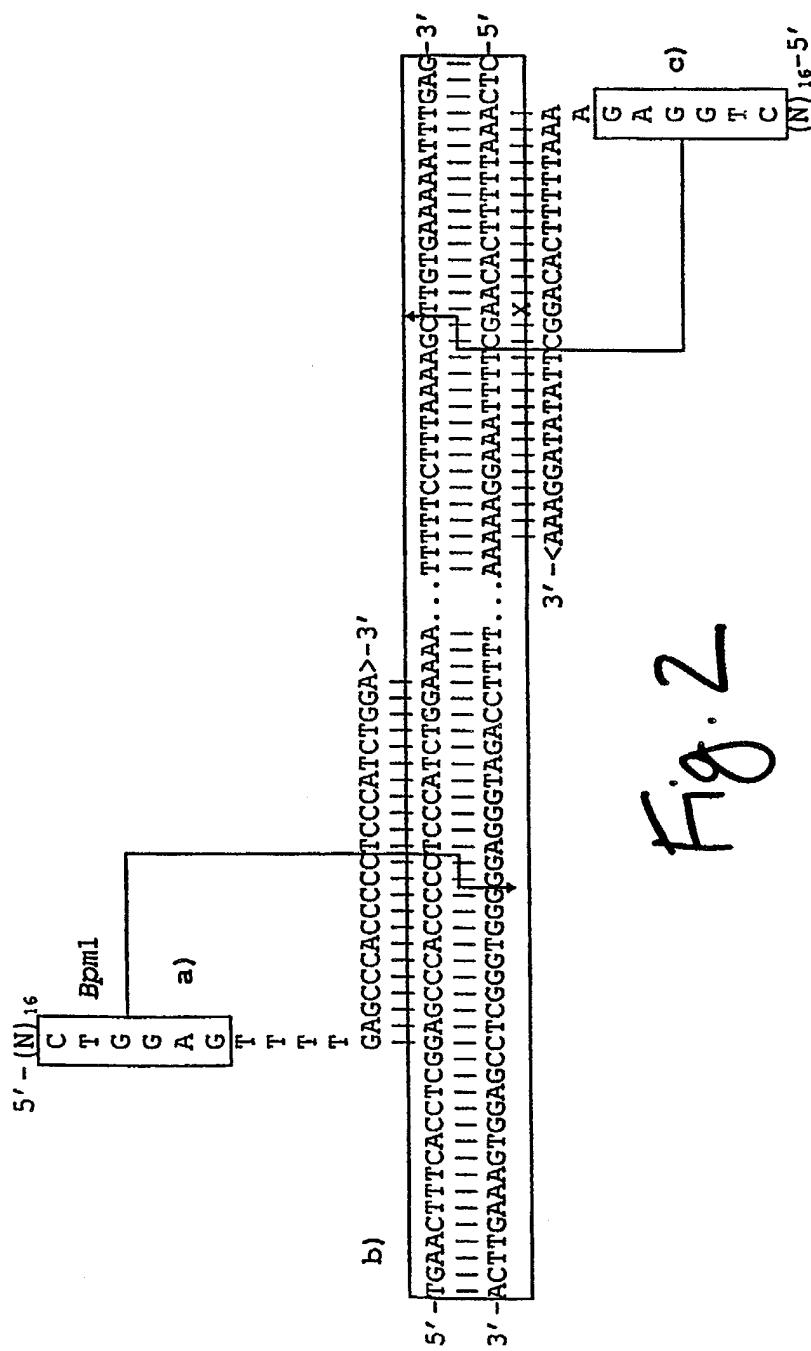


Fig. 3

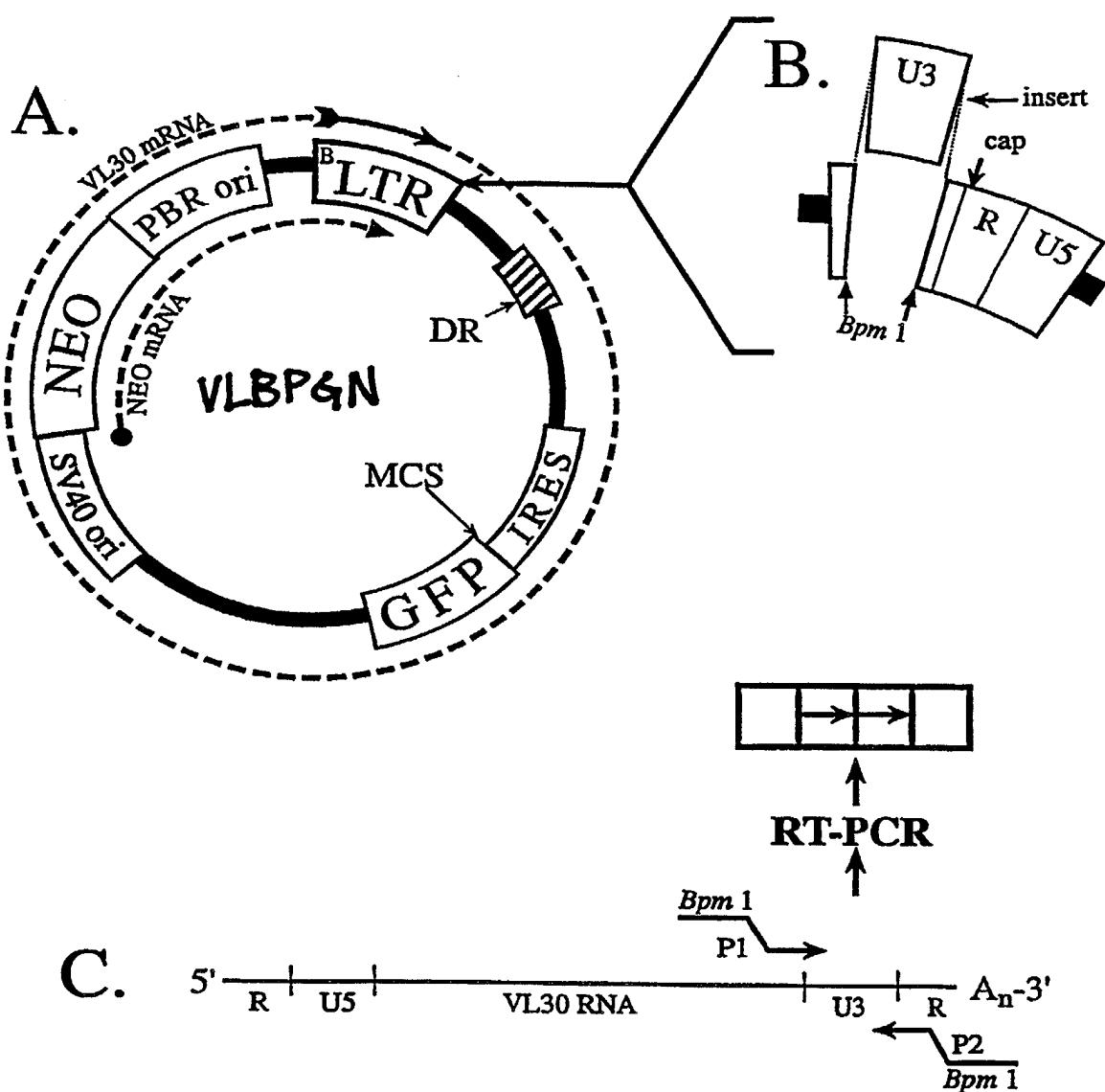


Fig. 4 A.

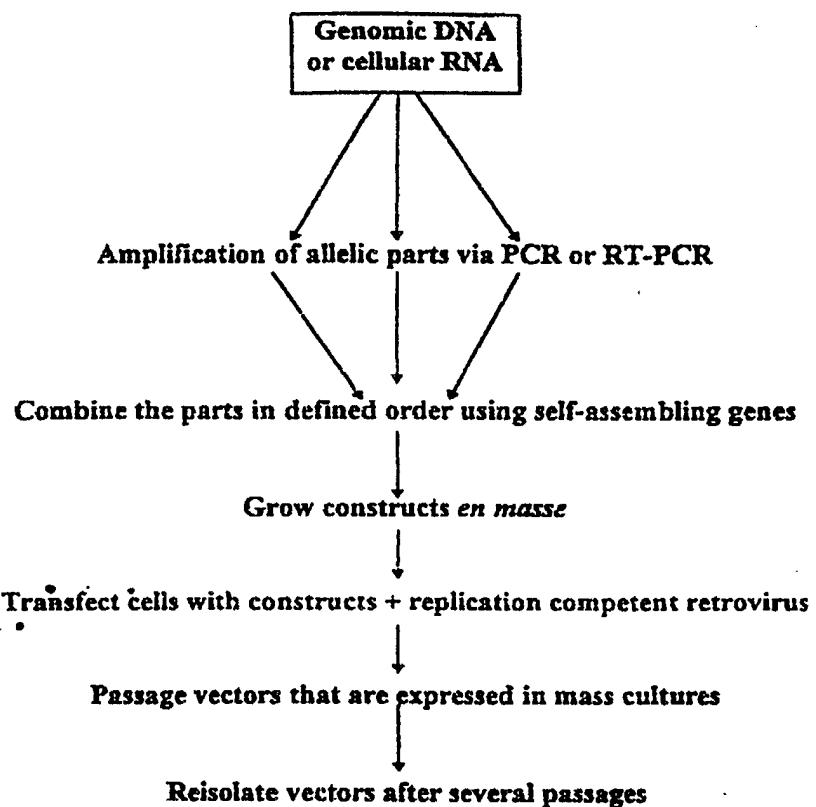


Fig. 4 B.

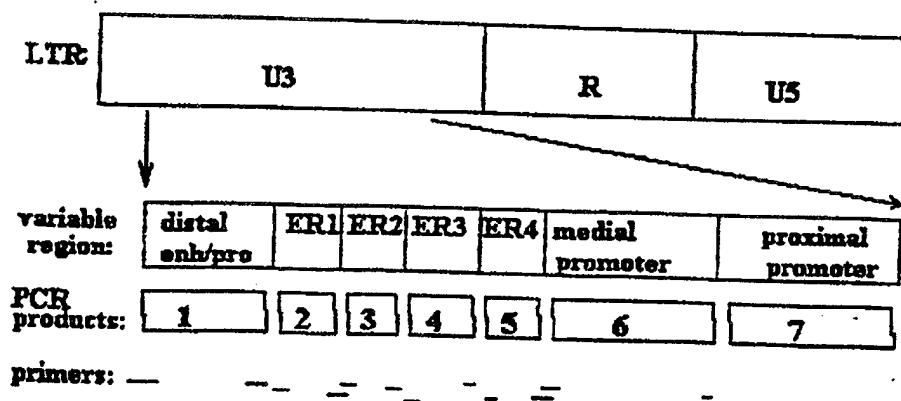


Fig. 5

1 I.D.2 CCTCCCAT AGGTTTTC CTCGGACAC TCCAAACTT TGCCTCCCA AACTCCCTAC CCTAAGTC GAAAGCTG TTCCAGAAC  
I.D.3 -----  
90 I.D.2 ATTTGAGA TAAGGCTC CTGACAAAC CTCGAATGA CTCGCAA TGTAGACA TGACTCTTA GTACGTTGG TTCCCTGATA  
I.D.3 -----  
180 I.D.2 GGATGACT CCTAGTAC GAGGTCCCT TGTAGACA TGTCTCTTA GTACGAAAC ATTTGAGA TAAGGCTC CTGAAAGAC CTCAAATCA  
I.D.3 -----  
270 I.D.2 GGACATGACT CCTAGTAC GAGGTCCCT TGTAGACA TGTCTCTTA GTACGAGA TTCCCTGGT AGAACTCCCT AGTGTGAA  
I.D.3 ACCAGGACT CCTAGTAC GAGGTCCCT TGTAGACA TGTCTCTTA GTACGAGA TTCCCTGGT AGAACTCCCT AGTGTGAA  
321 I.D.2 ACTTAACTT TCCCTCCA GTTCTCCCCC TTGAGTTT ACTTATAG C  
I.D.3 ACTTAACTT TCCCTCCA GTTCTCCCCC TTGAGTTT ACTTATAG C

Fig. 6

<p>1 CTCCTCCATC AGAGATTT CCCAGAAC TCTGAACTC TTACCCCG AGTGGATGCC AATGGATGCC TGAACCTTC ACCCTAGAGT TCGAACCCCTC          4 CTCCTCCATC AGAGATTT CCCAGAAC TCTGAACTC TTACCCCG AATGGATGCC TGAACCTTC ACCCTAGAGT TCGAACCCCTC          5 CTCCTCCATC AGAGATTT CCCAGAAC TCTGAACTC TTACCCCG AATGGATGCC TGAACCTTC ACCCTAGAGT TCGAACCCCTC          6 CTCCTCCATC AGAGATTT CCCAGAAC TCTGAACTC TTACCCCG AATGGATGCC TGAACCTTC ACCCTAGAGT TCGAACCCCTC          7 CTCCTCCATC AGAGATTT CCCAGAAC TCTGAACTC TTACCCCG AATGGATGCC TGAACCTTC ACCCTAGAGT TCGAACCCCTC          8 CTCCTCCATC AGAGATTT CCCAGAAC TCTGAACTC TTACCCCG AATGGATGCC TGAACCTTC ACCCTAGAGT TCGAACCCCTC          9 CTCCTCCATC AGAGATTT CCCAGAAC TCTGAACTC TTACCCCG AATGGATGCC TGAACCTTC ACCCTAGAGT TCGAACCCCTC          10 CTCCTCCATC AGAGATTT CCCAGAAC TCTGAACTC TTACCCCG AATGGATGCC TGAACCTTC ACCCTAGAGT TCGAACCCCTC          11 CTCCTCCATC AGAGATTT CCCAGAAC TCTGAACTC TTACCCCG AATGGATGCC TGAACCTTC ACCCTAGAGT TCGAACCCCTC          12 CTCCTCCATC AGAGATTT CCCAGAAC TCTGAACTC TTACCCCG AATGGATGCC TGAACCTTC ACCCTAGAGT TCGAACCCCTC          13 CTCCTCCATC AGAGATTT CCCAGAAC TCTGAACTC TTACCCCG AATGGATGCC TGAACCTTC ACCCTAGAGT TCGAACCCCTC</p>	90	<p>180</p> <p>91 CCAACTAAG ACTTTCGAA GAACTTTT GAGATAGGG CCTCTGGA CAACCTCGA ATGAACTCGA TACATTCGA AATAATAGGA          4 CCAACTAAG ACTTTCGAA GAACTTTT GAGATAGGG CCTCTGGA CAACCTCGA ATGAACTCGA TACATTCGA AATAATAGGA          5 CCAACTAAG ACTTTCGAA GAACTTTT GAGATAGGG CCTCTGGA CAACCTCGA ATGAACTCGA TACATTCGA AATAATAGGA          6 CCAACTAAG ACTTTCGAA GAACTTTT GAGATAGGG CCTCTGGA CAACCTCGA ATGAACTCGA TACATTCGA AATAATAGGA          7 CCAACTAAG ACTTTCGAA GAACTTTT GAGATAGGG CCTCTGGA CAACCTCGA ATGAACTCGA TACATTCGA AATAATAGGA          8 CCAACTAAG ACTTTCGAA GAACTTTT GAGATAGGG CCTCTGGA CAACCTCGA ATGAACTCGA TACATTCGA AATAATAGGA          9 CCAACTAAG ACTTTCGAA GAACTTTT GAGATAGGG CCTCTGGA CAACCTCGA ATGAACTCGA TACATTCGA AATAATAGGA          10 CCAACTAAG ACTTTCGAA GAACTTTT GAGATAGGG CCTCTGGA CAACCTCGA ATGAACTCGA TACATTCGA AATAATAGGA          11 CCAACTAAG ACTTTCGAA GAACTTTT GAGATAGGG CCTCTGGA CAACCTCGA ATGAACTCGA TACATTCGA AATAATAGGA          12 CCAACTAAG ACTTTCGAA GAACTTTT GAGATAGGG CCTCTGGA CAACCTCGA ATGAACTCGA TACATTCGA AATAATAGGA          13 CCAACTAAG ACTTTCGAA GAACTTTT GAGATAGGG CCTCTGGA CAACCTCGA ATGAACTCGA TACATTCGA AATAATAGGA</p>
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Fig. 7

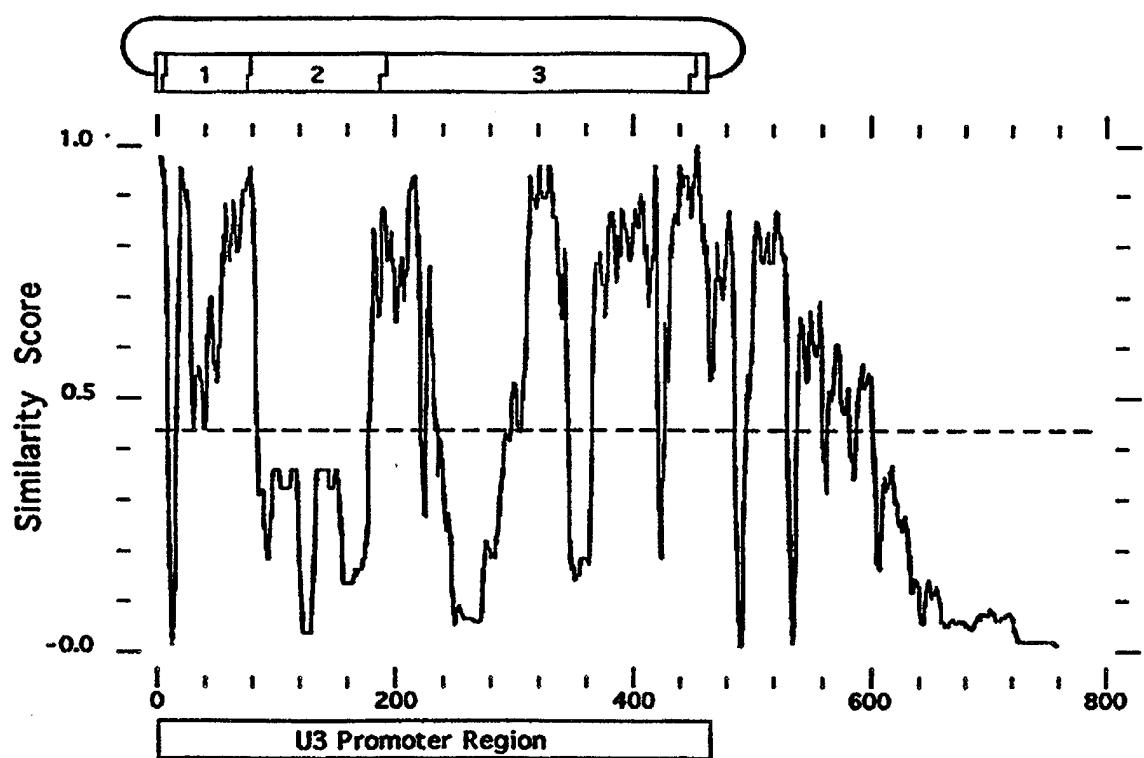
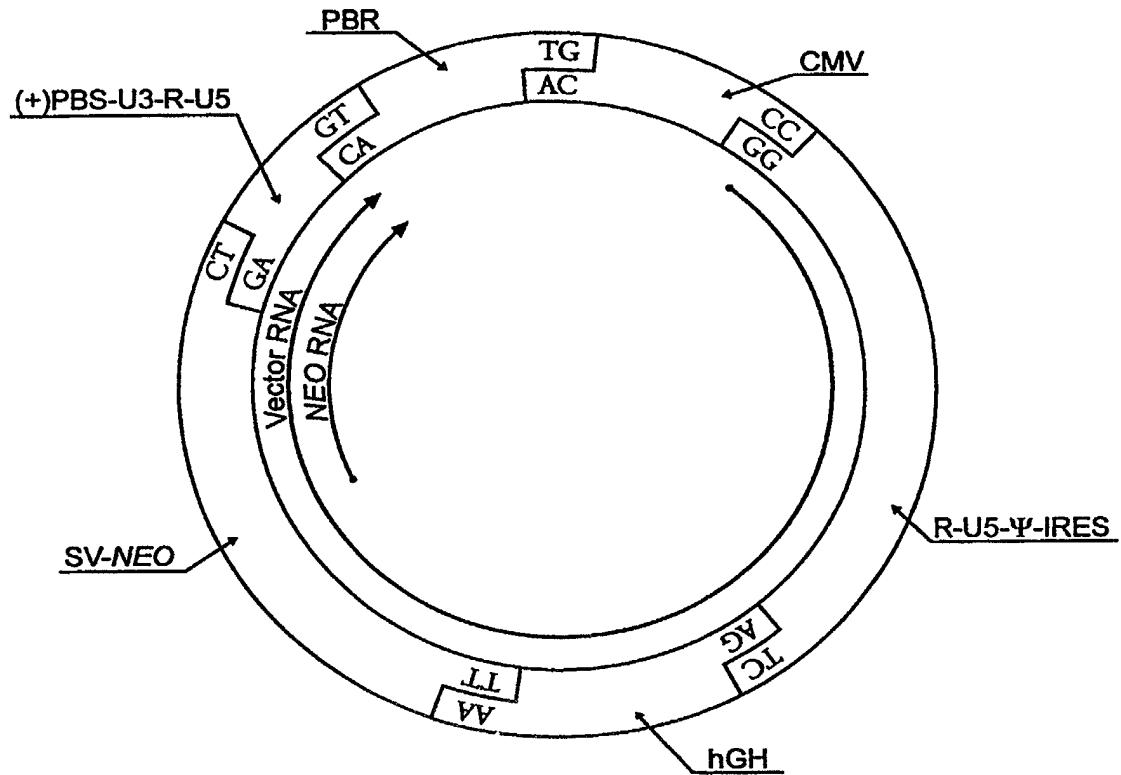


Fig. 8



# INTERNATIONAL SEARCH REPORT

I. International Application No  
PCT/US 98/03918

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C12N15/86 C12N15/10

According to international Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PADGETT K A ET AL: "Creating seamless junctions independent of restriction sites in PCR cloning" GENE, vol. 168, no. 1, 2 February 1996, page 31-35 XP004042930 see the whole document	1,2, 4-14, 19-21
Y	TOMIC, M. ET AL.: "A rapid and simple method for introducing specific mutations into any position of DNA leaving all other positions unaltered" NUCLEIC ACIDS RESEARCH, vol. 18, no. 6, 1990, OXFORD GB, page 1656 XP002069445 cited in the application see the whole document	3
Y	---	3
	-/-	

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

<sup>1</sup> Special categories of cited documents :

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Date of the actual completion of the international search

26 June 1998

Date of mailing of the international search report

09/07/1998

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Chambonnet, F

**INTERNATIONAL SEARCH REPORT**

In ational Application No
PCT/US 98/03918

**C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LEBEDENKO, E.N. ET AL.: "Method of artificial DNA splicing by directed ligation" NUCLEIC ACIDS RESEARCH, vol. 19, no. 24, 1991, OXFORD GB, pages 6757-6761, XP002069446 cited in the application see the whole document ---	1
A	CHAKRABORTY, A.K. ET AL.: "Synthetic retrotransposon vectors for gene therapy" FASEB JOURNAL., vol. 7, no. 10, July 1993, FOR EXPERIMENTAL BIOLOGY, BETHESDA, MD US, pages 971-977, XP002029486 see the whole document ---	1
P,X	WO 97 28282 A (STRATAGENE INC) 7 August 1997	1,2, 4-14, 19-21
P,Y	see the whole document ---	3
P,X	HODGSON, C.P. ET AL.: "Self-assembling genes (SAGE) : construction of complex vectors and combinatorial libraries without sub-cloning" CANCER GENE THERAPY, vol. 4, no. 6 conf. suppl.. November 1997. page s27 XP002069448 see the whole document ---	1
P,X	ZINK, A. M. ET AL.: "Transcriptional targeting with rescued LTRs : a hepatocyte promoter" CANCER GENE THERAPY, vol. 4, no. 6 conf. suppl., November 1997, page s28 XP002069449 see the whole document ---	22

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No  
PCT/US 98/03918

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9728282	A 07-08-1997	NONE	